

FUNCTIONAL CHARACTERIZATION OF
PROTEIN STABILIZED EMULSIONS

EVA TORNBERG
CIV.ING., ÖG

AVHANDLING FÖR TEKNISK DOKTORSEXAMEN

Lund 1978



**DIVISION
OF
FOOD TECHNOLOGY**



**CHEMICAL CENTER
LUND INSTITUTE OF TECHNOLOGY
LUND — SWEDEN**

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The emulsifying and interfacial properties of three food proteins represented by a soy protein isolate, a whey protein concentrate (WPC) and a sodium caseinate, have been studied.

In the interfacial tension studies an apparatus based on the drop volume principle has been used, and the kinetics of protein adsorption at the interface has been evaluated.

In the emulsification studies, preparation of emulsions has been performed in an isothermal, recirculating system. Power and energy consumption during emulsification have been measured and controlled. The emulsions formed were characterized in terms of creaming stability, fat particle size distribution and amount of protein adsorbed per unit area of fat surface.

Both the interfacial and the emulsification studies suggest that the caseinates mainly cover the interface by adsorption of casein monomers from the bulk, whereas the soy proteins migrate to the interface by the gross associated complex, which disintegrates at the interface in order to cover it. The whey proteins show an emulsifying behavior intermediate to that of the other two proteins.

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ERRATUM

- p. 5 line 15 ... as a function of the emulsification process... should be as a function of the variables that govern the emulsification process.
- p. 11 line 10 ... adsorbs at the interface at the same time ... should be adsorbs at the interface.
- p. 27 line 30 ... adsorbance ... should be absorbance.
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- Paper I p. 469
first paragraph ... protein (N x 6.25) 89.6% ... should be protein (N x 6.25) 93.4%.
second paragraph ... protein (N x 6.37) 89.3% ... should be protein (N x 6.43) 96.9%.
third paragraph ... protein (N x 6.25) 65.6% (dry wt), fat 1.9% ... should be protein (N x 6.55) 78.8% (dry wt), fat 5.4%.
- Paper I p. 472 line 2 ... flow conditions, effect and energy ... should be flow conditions, power and energy.
- Paper IV p. 14 line 10 ... the films start the penetration-controlled step ... should be the films the penetration-controlled step start.
- Paper IV p. 16 line 16 ... aggregates ... should be aggregated.
- Paper VI p. 8 line 7 ... roughly ... should be roughly.
- Paper VII p. 6 line 15 ... adsorbance ... should be absorbance.
- Paper VII p. 9 line 6 ... adsorbance ... should be absorbance.
- Paper VII p. 14 line 26 ... linear as a function ... should be independent.

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EVA TORNBERG

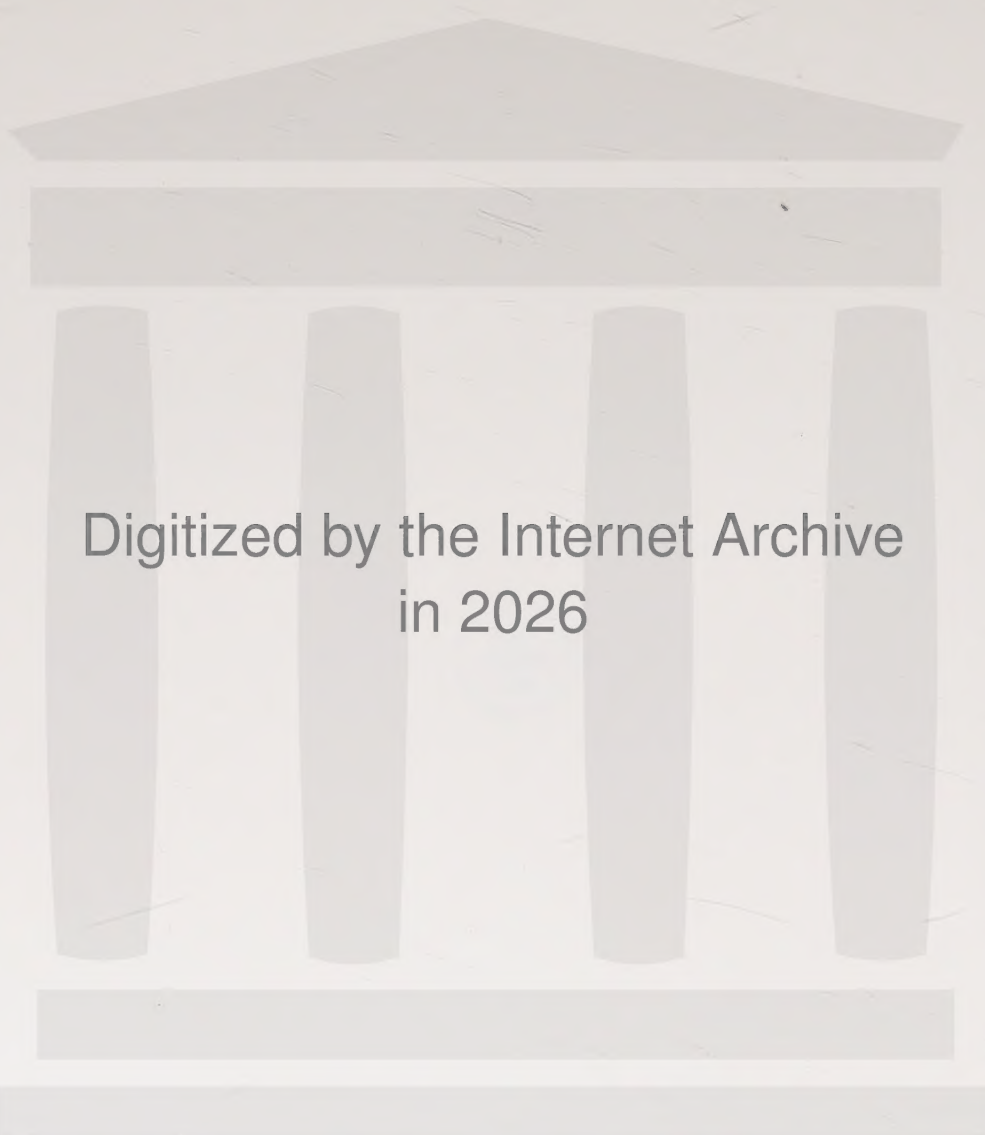
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Beginning to research,
is a beginning of a life-long romance.

Mark Tornberg



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FUNCTIONAL CHARACTERIZATION OF PROTEIN STABILIZED EMULSIONS

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This thesis is based on the following papers, referred to in the text by their Roman numerals:

- I E. Tornberg and A.-M. Hermansson. Functional Characterization of Protein Stabilized Emulsions: Effect of Processing. J. Food Sci. 42 (2) 468, 1977.
- II E. Tornberg. A Surface Tension Apparatus According to the Drop Volume Principle. J. Coll. Interface Sci. 60 (1) 50, 1977.
- III E. Tornberg. The Application of the Drop Volume Technique to Measurements of the Adsorption of Proteins at Interfaces. Accepted for publication in J. Coll. Interface Sci.
- IV E. Tornberg. The Interfacial Behaviour of Three Food Proteins Studied by the Drop Volume Technique. Accepted for publication in J. Sci. Fd Agric.
- V E. Tornberg and G. Lundh. Functional Characterization of Protein Stabilized Emulsions: Standardized Emulsifying Procedure. Accepted for publication in J. Food Sci.
- VI E. Tornberg. Functional Characterization of Protein Stabilized Emulsions: Creaming Stability. Accepted for publication in J. Food Sci.
- VII E. Tornberg. Functional Characterization of Protein Stabilized Emulsions: Emulsifying Behaviour of Proteins in a Valve Homogenizer. Submitted for publication in J. Sci. Fd Agric.

INTRODUCTION

The world population will continue to expand, and the need for protein in food will increase. Therefore, supplementary and new sources of proteins will need to be developed. During the last two decades much development has been achieved in the production of various non-conventional sources of protein. However, knowledge is still lacking as to supplement already existing foods, to replace or simulate traditional foods or to produce new foods out of these new raw materials. In connection with these requirements not only properties such as nutritional value and flavour of the protein product are important, but it should also possess certain functional properties. The functional properties of a protein product can be defined as those physico-chemical properties giving information about how the protein will act in a food system (1).

The difficulty to obtain relevant and basic information on functional properties of proteins is mainly due to the fact that a variety of empirical methods has been used, which makes it hard to compare published data on functional properties of different proteins. Moreover, in order to work out suitable methods for measuring the functional properties of proteins, the structures occurring in food such as emulsion, gel, foam and suspension, where the proteins take part, have to be considered. These structures can interact in a complicated way in the food system, and to settle out the problems, a scheme of investigations, as represented in figure 1, might be fruitful. There is a need for research to explain the physico-chemical mechanisms responsible for specific functional properties, and this research is represented as the biophysical background. The biophysical background will give knowledge of how proteins act in simple model systems of gels, emulsions, suspensions and foams. These model systems are then put together under controlled conditions to a model food system, where knowledge of the interactions between components and structures can be obtained. It is likely that this scheme of investigations would more successfully than the common trial-and-error technique provide us the tool for new product design, for the exchange of already existing proteins in food products and for the process optimization of production of protein products.

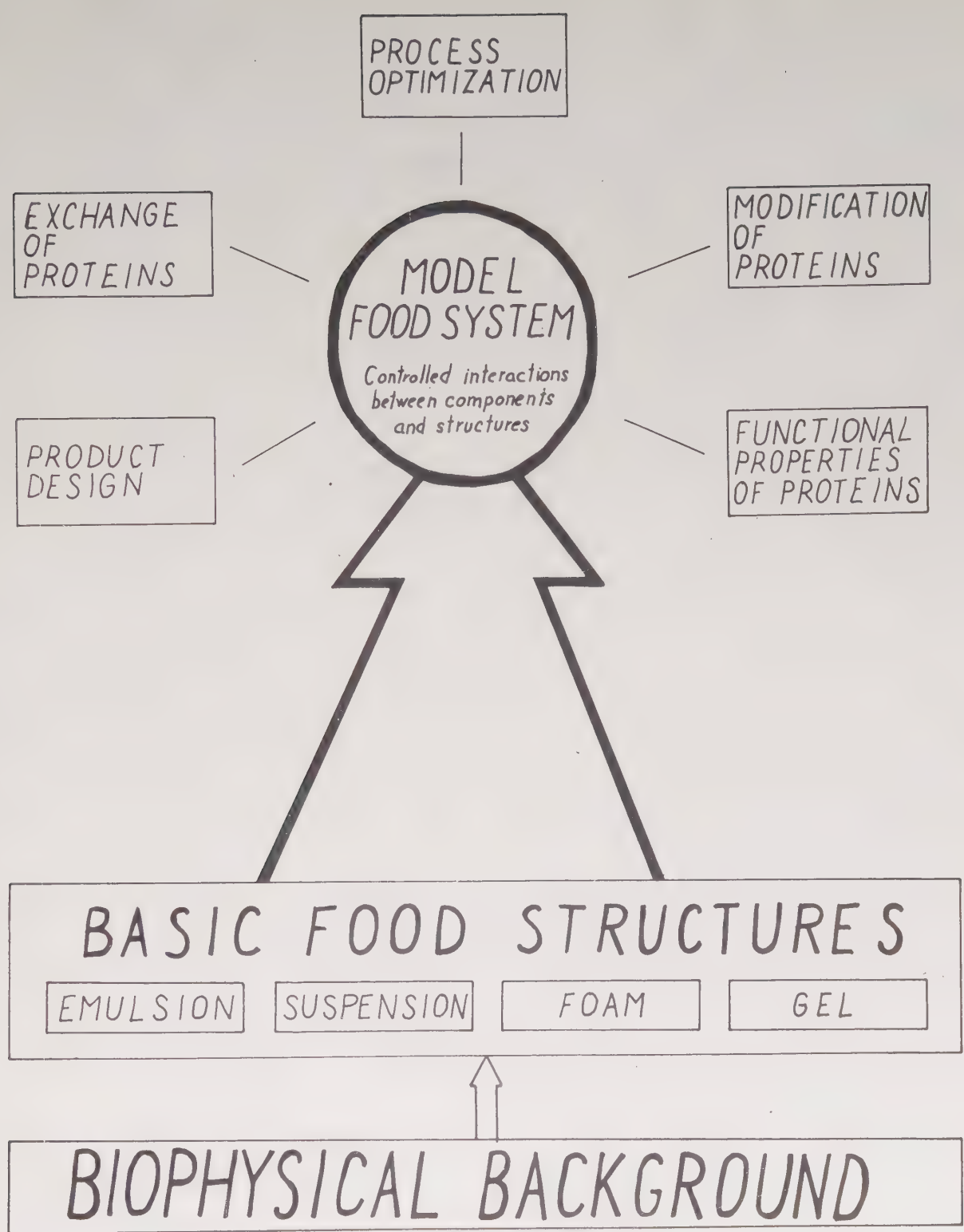


Fig. 1. A highly schematic scheme of investigations in order to obtain information about how a protein will act in a food system.

Concerning the suggested investigations, represented in figure 1, this thesis will be restricted to investigations of model systems of protein stabilized emulsions, and also to the interfacial behavior of proteins, giving some ideas of the biophysical background. The oil-in-water (o/w)-emulsion has been chosen as a model system as it occurs more frequently in food systems than the water-in-oil type.

Protein stabilized o/w-emulsions are found in various areas of the food industry. These include milk, cream, ice-cream, salad dressings, mayonnaise, sauces, meat emulsions, spray-dried emulsions and imitation milks. Emulsions in food can seldom be considered as "true" emulsions, since the fat can be plastic, and the food system may contain additional structures such as foam, suspension and/or gel. Moreover, "true" emulsions range from 3 up to 80% in oil phase volume and can differ in viscosity of the continuous phase. This leads to stability problems of different kinds. It is, therefore, difficult to develop a single test for measuring the emulsification properties of a protein.

Two main approaches have been used to study the emulsifying characteristics of proteins; emulsifying capacity and emulsion stability measurements. There is a great difference between the emulsifying capacity and the emulsion stability produced with proteins. The former measures the maximum oil addition until inversion or phase separation of the emulsion, whereas the latter measures the ability of the emulsion to remain unchanged. Instability of an emulsion can appear visually as creaming and fat separation. The underlying mechanisms of instability are flocculation and coalescence. Flocculation means collection of single emulsion droplets into aggregates, in which the membranes surrounding the fat droplets are intact, whereas coalescence involves destruction of the membrane material leading to larger fat droplets out of smaller. A comparison between methods so far used in emulsifying capacity and emulsion stability measurements is given in paper I.

It can be noted, when comparing the emulsifying properties of different proteins obtained by different authors (see the review of Kinsella (2)), how the results are influenced by the way the emulsions are made and on the conditions of measurements. The investigation presented in paper I, which is a study of the influence

of four different types of emulsifying equipment, as well as intensity and time of emulsification on the properties of the emulsions formed, further confirms this observation. It was found that these parameters profoundly affected the creaming stability of protein stabilized emulsions made up of 40 wt. % soybean oil and 60 wt. % protein dispersion of 2.5 wt. % protein content. Evidently, the properties of the emulsions formed are significantly determined by the kinetics of the emulsification process. This has also been demonstrated by Lankveld and Lyklema (3) for paraffin-in-water emulsions stabilized by a polymer, polyvinylalcohol. The pronounced effect of emulsification parameters on the properties of protein stabilized emulsion can probably be explained by the unusually high stabilizing power of the proteins, and by a mainly irreversible adsorption of proteins at the interface. The emulsifying behavior of proteins has therefore been studied as a function of the emulsification process. The emulsions formed have been characterized in terms of creaming stability, fat particle size distribution and amount of protein adsorbed per unit area of fat surface. These investigations (V.- VII) comprise the second part of this thesis. The first part (papers II - IV) comprises the interfacial behavior of the proteins as studied by the drop volume technique. In the 'general discussion' the results from the interfacial and the emulsifying behavior have been combined to give a more general view of proteins as emulsifiers.

B MATERIALS

B 1 Soybean oil

A commercially available soybean oil (AB Karlshamns Oljefabriker, Karlshamn, Sweden) was used. Analysis: fatty acid composition 18:2 54%, 18:1 23%, 16:0 10%, 18:3 8%; FFA 0.02% (w/w) (average molecular weight = 282). The amount of monoglycerides was so small as to be undetectable by thin layer chromatography. The content of soybean lecithin in the soybean oil must be well below 0.005%, as concluded from interfacial tension measurements. The interfacial tension decay between the soybean oil used and water after 40 minutes was $\approx 2.8 \text{ mN m}^{-1}$, whereas the interfacial tension depression after 40 minutes was $\approx 8 \text{ mN m}^{-1}$ when 0.005% soybean lecithin was added to refined and deodorized soybean oil.

B 2 Proteins

The proteins lysozyme, β -lactoglobulin and bovine serum albumin (BSA) were chosen in the interfacial tension measurements because they are well-defined proteins.

B 2.1 Lysozyme

Three times recrystallised and lyophilized egg-white lysozyme from Sigma Chemical Co. was used without further purification. Hen egg white lysozyme is a globular protein of molecular weight of 14 600, with 129 amino acids in a single polypeptide chain and four disulfide bridges (4). The isoelectric point is at pH 11. Sophianopoulos and van Holde (5) have concluded from sedimentation data that lysozyme is monomeric when $\text{pH} < 4.5$ (conc. $\leq 1.4\%$ and at 0.15 M KCl).

B 2.2 β -Lactoglobulin

Once recrystallised and lyophilized β -lactoglobulin (A and B) from Sigma Chemical Co. was used without further purification. β -Lactoglobulin has a molecular weight of 18 400, and is associated to form dimers at room temperature in solutions between pH 3 and 7 (6).

B 2.3 Bovine serum albumin (BSA)

Once recrystallised and lyophilized BSA from Sigma Chemical Co. was used without further purification. The molecular weight of BSA is approximately 66 000, and the isoelectric point is at $\text{pH} \approx 5$.

B 3 Protein products

Several new, non-conventional proteins have been introduced to the market, but the impact commercially has been meagre. Milk is still the major source of supplementary protein for the food industry, though soybean protein is becoming increasingly popular. A soy protein isolate, a sodium caseinate and a whey protein concentrate (WPC) were therefore chosen for this study. Moreover, the functional properties of these proteins depending on water interactions have been studied earlier (1).

The methods used in isolating and concentrating the protein products, such as isoelectric precipitation, spray drying and ultrafiltration, have been shown to essentially leave intact the functional properties of the protein products (2).

To be ascertain that during the emulsification process the protein itself was not severely affected, the protein dispersions used in this investigation were subjected to the same treatment as used for the emulsification procedures. The turbidity and the solubility of the dispersions were measured before and after treatment. No irreversible changes in the properties of the protein dispersions were observed.

B 3.1 Soy protein isolate

The production of the soy protein isolate is described in papers IV, VI and VII. The same batch was used throughout the work. Analysis: protein (N x 6.25) varied from 99.4 to 96.0% (dry weight) during the course of the investigations.

The soy proteins consist mainly of four protein fractions, where the two major fractions, the 7S and 11S, constitute two thirds. The 7S and 11S globulins account, respectively, for most of the proteins in each fraction. The 7S globulin consists of at least 9 subunits, where each has a molecular weight of 22 000 - 24 000, giving a particle weight of 180 - 210 000 (7). The 11S protein has a particle weight of 309 - 363 000, where six acidic subunits each have a molecular weight $\approx$ 35 000, and six basic subunits have each a molecular weight of $\approx$ 20 000 (8). The contents of α -helix, β -structure and random coil in both the 7S and 11S globulins is estimated to be approximately 5, 35 and 60%, respectively (9). The tertiary structure of the proteins in the 7S and 11S globulins appears to be a compact structure stabilized by hydrophobic bonds (10), but this folded structure has been proved to be easily destroyed upon dissociation of the proteins into subunits (11).

Around pH 7 and at very low ionic strength ($\approx$ 0.01) dissociation occurs for the 7S and 11S globulins (10). When increasing the ionic strength to 0.1 both the 7S and 11S globulins start to

associate, but with a further increase of the ionic strength up to 0.5 dissociation occurs once more to the monomeric form (10, 12).

B 3.2 Caseinate

Spray blend caseinate (DMV, Holland), a commercially available sodium caseinate from the same batch, was used. Analysis: protein ($N \times 6.43$) varied from 96.9% to 92.6% (dry weight) during the course of the investigations.

The native casein 'micelle' subunit is considered to have a particle weight of approximately 250 000, each subunit containing about 12 molecules with a mean composition of 50% α_s -casein, 30% β -casein and 15% κ -casein (13). The major component of α_s -casein is α_{s1} -casein, and it has a molecular weight of 23 500. The molecular weight of β -casein is around 24 000 and that of κ -casein approximately 19 000. β -Casein in solution is reported to be best described as a random coil, and similar descriptions hold for α_{s1} - and κ -caseins, though to a lesser extent (13). The casein molecules can be characterized as being hydrophobic, and they also show pronounced tendencies to associate in water solutions. α_{s1} - and β -caseins associate together to form a complex which is more stable than the complexes formed by self-association of either of these two proteins (14). The α_{s1} - κ -casein complex is, however, most stable (14).

Schmidt and Buchheim (15) have suggested that the preparation of sodium caseinate destroys the original properties of the casein 'micelle' and possibly also the structure of the submicelles. Some other observations, however, made by Creamer and Berry (14) have shown that complete disaggregation of the caseins did not alter their subsequent ability to form subunits, which were indistinguishable from those obtained directly from casein micelles by gel filtration.

B 3.3 Whey protein concentrate (WPC)

The preparation of the WPC is described in papers IV, VI and VII. The same batch has been used throughout the work. Analysis: protein ($N \times 6.55$) varied from 69.8% to 67.8% (dry weight) during the course of the investigations. The lipid content was found to be 7.6% (dry weight).

Most of the lipids were removed by precipitation at pH 4.5 and a following centrifugation. The supernatant was checked in interfacial behavior to that of the original WPC, and the interfacial tension decay of the former was not distinguishable within the limits of error from the latter. Furthermore, SDS-electrophoresis of the supernatant has shown that it mainly consists of the two major proteins of the WPC, β -lactoglobulin and α -lactalbumin, and so it was concluded that these two proteins mainly contribute to the interfacial and emulsifying behavior shown by the WPC.

A whey protein concentrate consists of $\approx 59\%$ β -lactoglobulin and $\approx 22\%$ α -lactalbumin, with relatively small quantities of serum albumin, immunoglobulins and caseins, mainly κ -casein. Data available on β -lactoglobulin has been given above. α -Lactalbumin has a molecular weight of 14 200. Between pH 5.4 and 9 α -lactalbumin has a rather stable conformation and appears in a monomeric form (6).

C DISCUSSION OF THE EXPERIMENTAL TECHNIQUES AND THE RESULTS

C 1 Interfacial behavior

C 1.1 Experimental techniques

When an air/liquid or liquid/liquid interface is first formed a finite time is required to establish equilibrium in the surface phase. If this time is short, as in the case of pure liquids and salt solutions, the final value of surface tension (γ) is immediately obtained. The registration of γ is in those cases time independent, whereas for solutions of components with higher molecular weight, such as proteins, the equilibrium value of γ is attained only after a readily measurable time, i.e. γ is time-dependent.

Different methods of measuring time-independent interfacial tensions are compared in paper II. The main objection against the frequently used Wilhelmy plate method arises from problems concerning the contact angle. The drop volume and the pendent drop methods do not suffer from this disadvantage. The accuracy of the latter method is, though, limited (16). The drop volume

method has been chosen, and a convenient apparatus based on this principle has been designed, which is described in paper II. The procedure for measuring time-independent interfacial tensions is given in the same paper. Briefly it involves the formation of a drop until it detaches, and the measurement of the volume of that part of the drop that falls.

No comparison between different methods in measuring the interfacial tension decay of polymer solutions, like protein solutions, has appeared in the literature. In paper III an investigation has been performed, in which the lowering of the interfacial tension of lysozyme, β -lactoglobulin and BSA has been recorded with both the drop volume method and the Wilhelmy plate technique. In order to measure time-dependent interfacial tensions with the drop volume method a modification of the procedure reported in paper II was needed. A drop of a certain volume, corresponding to a certain γ -value, is expelled rapidly, and the time necessary for the surface tension to fall to such a value that the drop becomes detached is measured. Part of the total drop does not fall, and the remaining portion will have its surface tension altered by adsorption of surface active material. Hence, this aged surface must be eliminated when the next drop is to be formed. Thus, by forming one or two drops rapidly, the next drop to be measured should be free of aged surface.

The drop volume apparatus constructed was proved to be successful in determining the interfacial tension between a variety of pure liquids, covering interfacial free energies from 70 down to 10 mN m^{-1} (II). Temperature-dependence of the surface tension of water between 20 and 50°C was also easily recorded (II). The time-dependent interfacial tension measurements in paper III indicated that slower kinetics of all the protein solutions studied were obtained with the drop volume method compared to the Wilhelmy plate technique. This observation could probably be attributed to the enlargement of the surface of the drop throughout the process of the surface tension decay in the drop volume method. Another interesting observation was that the proteins investigated were differently sensitive to this surface expansion.

C 1.2 Theory applied to the kinetics of protein adsorption

The adsorption of a macromolecule, such as a protein, at an interface can be expected to differ from that of an ordinary low molecular weight compound, as adsorption must take place in steps corresponding to molecular segments, and if one segment of the molecule adsorbs onto a surface, the probability of the adsorption of neighboring segments will be greatly increased. This will probably lead to irreversible adsorption of the protein, due to the possibility that more than one segment from the same molecule adsorbs at the interface at the same time.

The rate of reduction of interfacial tension can be considered as due to three consecutive or concurrent processes (17, 18): diffusion of whole protein molecules to and attachment at the interface; spreading or unfolding of separately distributed adsorbed molecules; molecular rearrangements of adsorbed molecules within the interfacial film.

In paper III the kinetics of protein adsorption at an interface has been analyzed in terms of distinguishable rate-determining steps. This analysis can shortly be summarized as follows. Diffusion is considered to be the rate-determining step as long as a plot of the interfacial tension against $t^{1/2}$ is linear. When a sufficiently high adsorption energy barrier exists, the diffusion will no longer be rate-determining, but the rate of protein penetration into the interface will be rate-limiting. This barrier consists of the work done ($\Pi \Delta A_{ag}$) in creating an area ΔA_{ag} in a surface film of surface pressure Π in order to adsorb an active group (ag) in the protein molecule. This situation is assumed to prevail if a plot of $\ln \frac{d\Pi}{dt}$ versus Π is linear, where the slope gives ΔA_{ag} . When $\ln \frac{d\Pi}{dt}$ is independent of Π , i.e. when $\Delta A_{ag} \approx 0$, this is taken as an indication of a rearrangement process taking place, in which no new area, ΔA_{ag} , has to be created in order to adsorb protein segments. The surface tension is, however, still lowered by protein segments being adsorbed at the surface unoccupied by protein segments in the preceding processes.

Diffusion controlled adsorption of proteins at an interface can imply either that the molecules diffuse to the interface and adsorb without further spreading, or that spreading or unfolding is so

rapid that diffusion becomes the rate-controlling factor. The penetration step may involve either spreading or unfolding of already adsorbed molecules, or adsorption of additional molecules arriving at the interface. In order to be adsorbed, however, a surface area, ΔA_{ag} , has to be cleared in the film, which requires molecular rearrangements of the already adsorbed protein molecules. The larger the surface area, ΔA_{ag} , to be cleared, the greater the number of residues to be rearranged within the surface film and the slower is the process.

C 1.3 Interfacial behavior of single proteins

So far a considerable amount of work has been devoted to the study of spread protein films, which are formed by adding a known amount of protein directly to the water surface. However, the study of the adsorption of proteins at the interface from a subphase of known concentration is more relevant to what really happens during the emulsification process. Moreover, it has been pointed out by van den Tempel (19) that it is not so much the absolute value of γ which is crucial, but rather the gradients in γ that arise during the emulsification process. The kinetics of adsorption are therefore a particularly interesting subject for study. Furthermore, it is not only the kinetics of adsorption that is of relevance, but also the concurrent extension of the interface, when considering the emulsification process. Therefore it seems reasonable to assume that the measurement of the interfacial decay of protein solutions as a function of time with the drop volume method, gives relevant information on the interfacial behavior of proteins during emulsification.

When protein molecules have a net charge, the net rate of collisions with the interface (Z) will be hindered by the electrostatic repulsion during adsorption. Due to the intramolecular electrostatic repulsion when charged, flexible protein molecules become more expanded in bulk, which will result in a lower diffusion coefficient. Graham (17) argues that the ease of penetrating a protein surface film, and the number of successful collision resulting in the molecule being adsorbed (Z) relate to the hydrophobicity of the protein molecule. Increasing tendency to unfold at interfaces with increasing hydrophobicity of the proteins has been

observed by Birdi (20) for the adsorption of several proteins at the air-water interface. Proteins with a high molecular flexibility in the bulk medium have a high surface activity (17, 21). The rate of rearrangements taking place in a protein film has been shown to be strongly dependent on the compressibility of the adsorbed protein film (17).

The interfacial behavior of three proteins, lysozyme, β -lactoglobulin and BSA, has been investigated at a protein concentration of $10^{-2}\%$ (w/w) and at pH 7 and an ionic strength of 0.1 (III). The kinetic evaluation of the γ -t-curves gave the following results.

The diffusion of β -lactoglobulin is most rapid, followed by BSA and lysozyme, the latter diffusing comparatively slowly. This seems to be contradictory, because the molecular weight of lysozyme is small and probably no association in the bulk occurs under the conditions used. As lysozyme is far away from the isoelectric point at the pH employed, the net charge of the molecule is high; hence the electrostatic hindrance during adsorption will probably contribute to a diminished number of successful collisions. This is probably one of the reasons why diffusion of lysozyme is relatively speaking so slow. Evidently, the probability of successful collision of molecules with the interface is of great importance in making the diffusion-controlled process fast, which also has been reported by Graham (17). For the surface tension decay of lysozyme, an initial period before the diffusion-controlled step has been observed, where the rate of arrival of protein segments or molecules at the surface, as reflected in $\frac{d\Pi}{dt}$, increases as a function of Π . This phenomenon has been attributed to the enlargement of the surface of the drop, accomplished by the surface tension decay of the first arrived molecules, being faster than the process of building up an activation barrier against further adsorption, which is also a consequence of the surface tension decay. The probability of this surface enlargement effect probably increases, when the rate of diffusion is diminished, which seems to be in accordance with the observations made for lysozyme.

The different sensitivity of the three proteins to the surface enlargement in the drop volume method, reflects their differing ways of covering the interface. Probably due to the surface renewal during the surface tension decay in the drop volume method, the diffusion step is slowed down, and the unfolding step is promoted. Both these phenomena enhance the possibility of concurrent unfolding or spreading of adsorbed molecules during the diffusion-controlled step, when measuring with the drop volume method, which will lead to build up of an adsorption barrier at an earlier stage of the adsorption process, i.e. at a lower Π . This reasoning will hold as long as unfolding or spreading of molecules gives rise to lowered compressibility of the surface film. The γ -t-curve of β -lactoglobulin obtained with the drop volume method is not so different from the curve obtained with the Wilhelmy plate method. This can probably be due to the diffusion of β -lactoglobulin being so rapid that it does not slow down much, when measuring with the drop volume method. For BSA the interfacial tension decay is diffusion-controlled to a higher surface pressure Π , and is faster, when using the Wilhelmy plate than the drop volume method, which is in accordance with the reasoning above. For lysozyme an earlier start of the penetration-controlled step is not observed when measuring with the drop volume method, which can be attributed to its low capability to unfold at an interface.

The concentration dependence of the surface tension of 1 M KCl solutions of lysozyme has been investigated. The most striking feature to be observed is a faster change in, as well as a larger lowering of, the surface tension with increasing bulk concentration. The kinetic evaluation of the γ -t-curves, gave the following characteristics. At lower bulk concentrations (concentrations $\leq 0.01\%$ (w/w)) the diffusion step is slowed down, so that the probability of the surface enlargement effect increases. The results indicate that the lysozyme molecules spread more easily during the diffusion step at lower bulk concentrations. This is in accordance with Adams et al. (22), who deduced from their results with lysozyme films that low bulk concentration favours the formation of an adsorbed film containing a mixture of unfolded and native molecules, while a high concentration leads to the formation of a film of mostly native molecules.

C 1.4 Interfacial behavior of protein products

The study of the interfacial tension decay of complex protein products has been very restricted. The only study on the surface behavior of soy proteins which has so far appeared in the literature has been on the film formation of soy proteins during heating, when making so called yubas (23). The surface behavior of the different caseins and the major whey proteins, β -lactoglobulin and α -lactalbumin, has been studied previously. References to these studies are given in paper IV. Mitchell et al. (24) have studied the adsorption behavior of both the caseins and the whey proteins at pH 7 and ionic strength of 0.1 at the air-water interface with the Wilhelmy plate method. The work suggests the following order of the surface activity of the proteins: β -casein [22] > α_1 -casein [17] > κ -casein [15] > β -lactoglobulin A [12.5] > β -lactoglobulin B [11] > α -lactalbumin [10]. The number within the brackets denote the surface pressure in mN m^{-1} attained after 50 minutes with 0.001% initial protein concentration in the bulk phase.

The present protein products have been investigated, when dispersed in distilled water and in 0.2 M NaCl solution at pH 7, denoted as (0 - 7) and (0.2 - 7), respectively.

The time- and concentration-dependence of the interfacial tension of the soy protein isolate, the WPC and the caseinate at the air-water interface have been recorded with the drop volume method (IV). The concentration dependence of the interfacial tension of the three food proteins can be followed in figure 2, where the surface pressure attained after 40 minutes, $\Pi_{40 \text{ minutes}}$, is plotted against the initial subphase concentration. At high concentrations ($10^0 - 10^{-1}$ wt. % of initial subphase concentration), the surface activity of all the proteins is high and almost equal, whereas at medium concentrations ($10^{-1} - 10^{-3}$ wt. %) the differences in surface behavior of the proteins become evident. The caseinate (0.2 - 7) system is most effective as a surface active agent and is more or less independent of concentration within this concentration range. The opposite behavior is observed for the soy protein substrates, which gradually lose their surface activity with decreasing subphase concentration. WPC (0 - 7) and caseinate (0 - 7) have a rather similar concentration-dependence in this range, and the curves are in between those of the caseinate (0.2 - 7) and the soy proteins. The addition of salt (0.2 M NaCl) to the WPC-dispersions raises the surface activity of the WPC

not far beyond that of the caseinate (0.2 - 7). The increase in lowering of interfacial tension due to salt addition is also observed for the other two proteins. At very low concentrations, the caseinates drastically lose their surface activity and the soy proteins have no discernible interfacial depression effect, and thus the whey protein concentrates, which lose their surface activity less abruptly, are superior in this concentration region.

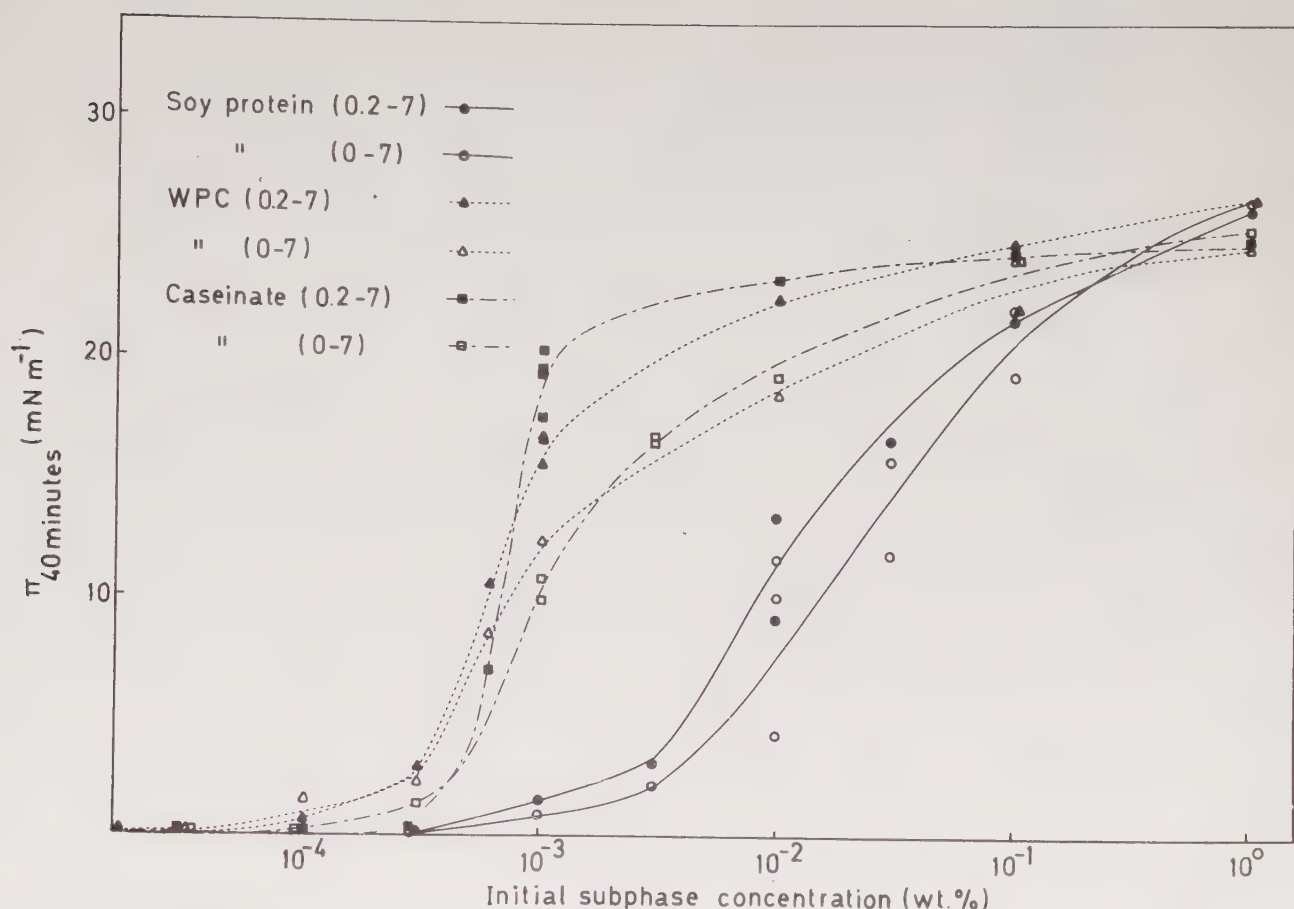


Fig. 2. The surface pressure attained after 40 minutes, $\pi_{40 \text{ minutes}}$, as a function of the initial subphase concentration for all the proteins studied at different ionic strengths (IV).

From the kinetic analysis of the γ -t-curves of the three food proteins at different ionic strengths and concentrations, the following interfacial behavior of the proteins was suggested.

Similar concentration-dependence phenomena have been observed for the three protein systems, i.e. diffusion is slower, the penetration step starts at a lower π , and ΔA_{ag} is larger at the same π for decreasing concentration. The lower rate of diffusion

for decreasing concentration allows the proteins to spread more easily during the diffusion step, thereby providing an adsorption barrier against further adsorption at a lower Π , which is also observed. The interfacial tension decay is slowed down at lower concentrations, due to both the reduced diffusion rate and the enhanced number of residues (larger ΔA_{ag}) participating in the rearrangement processes during the penetration-controlled steps compared at the same Π .

The soy proteins diffuse slowly to the interface in comparison with the other two proteins, which indicates that the soy proteins migrate to the interface with the gross quaternary structure intact. The rate-determining step of penetration of molecules or segments into the film starts at a relatively low surface pressure, especially in 0.2 M NaCl, compared to the other proteins. The adsorption of soy protein (0 - 7) at an interface is more diffusion-controlled, and this step persists for longer periods of time than for soy protein (0.2 - 7). This may seem to be contradictory, because the soy protein is aggregated more in 0.2 M NaCl solution than in distilled water. The anomaly might be explained, partly, by a greater Z-value in the case of the soy protein (0.2 - 7), due to reduced electrostatic repulsion during adsorption, and partly, by the higher spreading ability of the soy protein (0.2 - 7) at the surface (IV).

The whey proteins diffuse quickly to the interface, which is in accordance with their aqueous association; mainly small molecules and molecular complexes. The WPC (0 - 7) diffuses somewhat slower than WPC (0.2 - 7) to the interface, which is most evident in the observation that the effect of surface enlargement occurs at a higher concentration for WPC (0 - 7). This might be due to a lower Z-value for WPC (0 - 7) than for WPC (0.2 - 7). WPC (0.2 - 7) covers the interface to a higher degree by diffusion than WPC (0 - 7), which indicates that WPC (0 - 7) spreads or unfolds more easily at the interface.

Although the caseinate has a complex quaternary structure, like the soy proteins, it has a very different surface behavior. The diffusion step is almost as rapid as that of WPC at concentrations above

10^{-3} wt. %. This can be explained if the migration to the interface of the caseinates is performed mainly by the free casein molecules, which are in equilibrium with those in the submicelles. The results obtained from the work by Creamer and Berry (14) show that the casein 'micelle' subunits are in equilibrium with their component caseins. At a concentration of 10^{-3} wt. % the rate of the diffusion step is drastically slowed down, which was assumed to be due to the fact that the migration of the casein molecules (preferentially β -casein) from the interior of the submicelle is the rate-controlling factor at this low concentration (IV). The diffusion-controlled occupation of the interface is very evident for the caseinates, especially at an ionic strength of 0.2, compared to the other proteins. The present results indicate that the very flexible, random coil-like casein molecules have simpler kinetics than the other proteins studied, involving diffusion to the interface and direct anchoring of the freely available hydrophobic segments. Due to higher electrostatic repulsion between adsorbed species at the interface for caseinate (0 - 7) compared to caseinate (0.2 - 7), the caseinate (0.2 - 7) molecules probably can more densely pack at the interface. This will give rise to an adsorption barrier at a higher Π for caseinate (0.2 - 7), which also has been observed. The reduced speed of the diffusion process of the caseinate (0 - 7) as compared to caseinate (0.2 - 7), might be explained by an enhanced electrostatic repulsion during adsorption for caseinate (0 - 7) and thereby a reduction of Z .

To summarize what has been learnt about the interfacial behavior of the three food proteins investigated, a highly schematic representation is given in figure 3.

C 2 Emulsifying behavior

C 2.1 Emulsifying techniques

The common method of preparing emulsions is to apply a force to break up the interface into globules. Deformation and disruption of globules are caused by pressure or velocity differences in the liquid, and several mechanisms are possible (25). The most relevant mechanisms are shear, turbulence and cavitation, which are

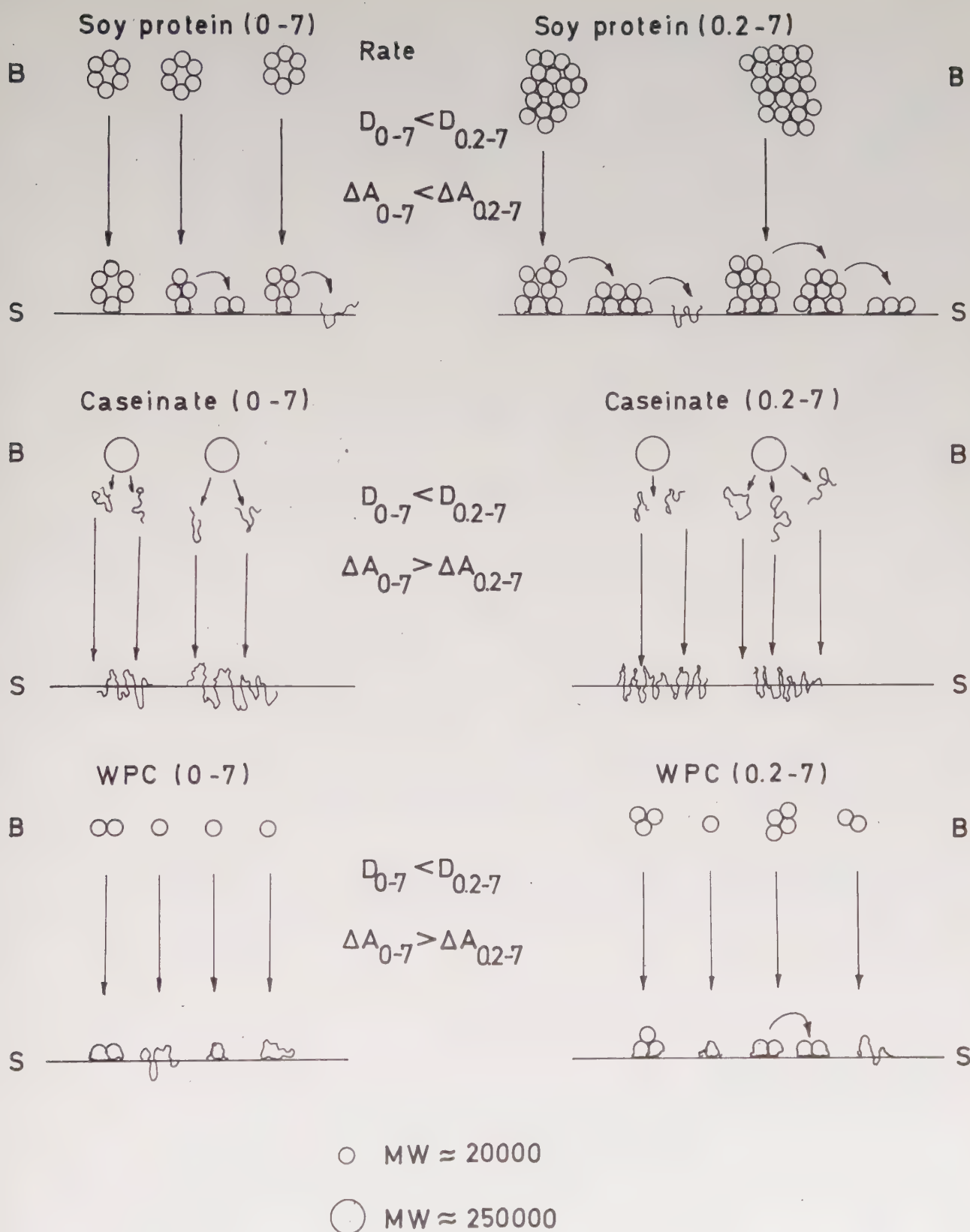


Fig. 3. A highly schematic representation of the differing interfacial behavior of the three food proteins at different ionic strength.

B : in the bulk medium

S : at the interface

D : diffusion rate

ΔA : is assumed to be inversely related to the rate of rearrangement in the protein film

illustrated in figure 4. Shear causes elongation of the droplet until it disrupts into two globules. Most of the energy in turbulent flow is dissipated in small eddies, which cause local high pressure gradients effective in disrupting emulsion globules of the same order of size (25). The mechanism of disruption in cavitation is very like that of turbulence (25). Cavitation is the formation and collapse of small cavities in a liquid. Collapse causes high pressure gradients, which lead to fat globule disruption.

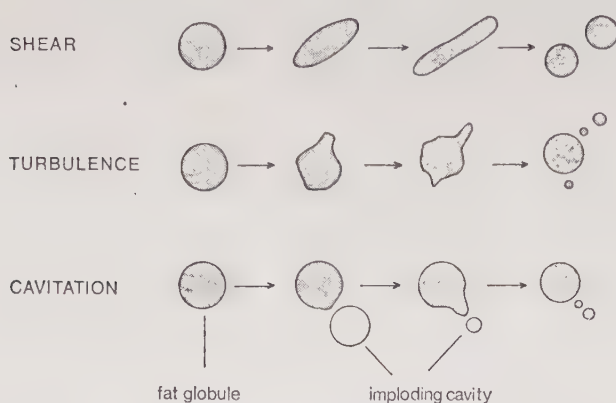


Fig. 4. A schematic representation of different mechanisms of globule disruption (25).

Simple mixers, turbo-mixers, colloid mills, pressure homogenizers and ultrasonic devices are the main tools for emulsification in the food industry.

Mixers are used for premixing the ingredients in the production of salad creams, artificial creams for cake filling, and are furthermore used in the production of mayonnaise, processed cheeses and many other food emulsions. They have a rather poor efficiency, giving globules of the order of 10 μm diameter (25). The prevailing mechanism of emulsification is supposed to be shear (25).

The turbomixer or ultra-turrax is claimed to be more effective than the simple mixer, since cavitation as well as turbulence are assumed to be produced (25). But a very wide spread in globule size around 5 μm is obtained, and the energy consumption is excessive (25). The active part of a turbomixer consists of

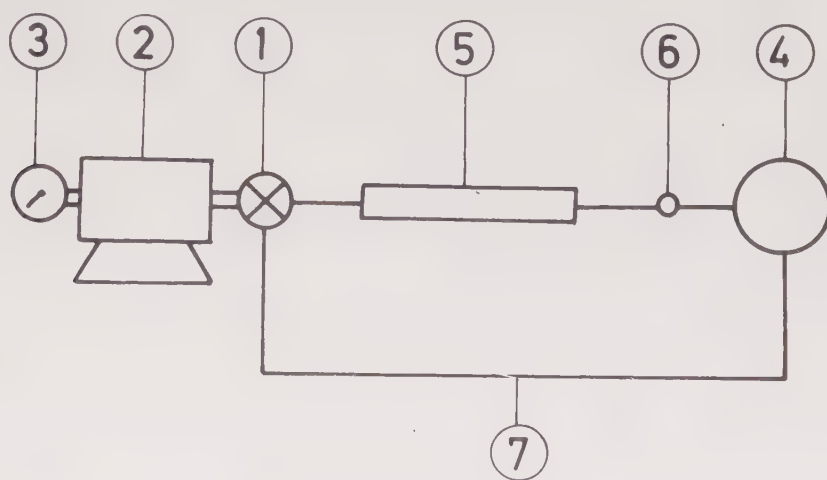
a stator and a rotor, which are both toothed and separated by a small clearance. The liquid circulates through the impellers due to the pumping action exerted by the rotor; it is sheared between both impellers and it impinges upon the slots in them.

Colloid mills are most suitable for making very viscous emulsions. In a colloid mill emulsification of the liquid is carried out under strong shearing flow in a narrow gap between a high speed rotor and a stator surface with no slots. Because of the high speed and the narrow gap, very strong shear flows are set up, and particle diameters of the order of 2 μm are easily obtained (26).

Valve homogenizers are widely used in the milk industry for homogenizing milk products. They are also used for many other emulsion-based food products. A valve homogenizer is a device in which emulsification is achieved by forcing the liquids through a small valve opening with a large pressure drop. This results in particle sizes of about 1 μm with a comparatively narrow size distribution (25). Mulder and Walstra (25) claim that at least in larger machines globules are mainly broken up by small turbulent eddies. According to Kiefer (27) and Treiber (28) the homogenizing efficiency of a valve homogenizer is mainly attributed to the amount of cavitation occurring. Phipps (29) proposed that it is a viscous shear type of break-up in the elongational flow at the entrance of the valve that causes disintegration into smaller droplets.

There are many applications for ultrasonic homogenizers in the food industry, including the manufacture of salad creams, ice-cream, cream soups, artificial creams and baby foods, but they are rarely used. A sonifier disruptor horn generates ultrasonic waves, which produce intense cavitation in the liquids and thereby disruption. Very small globules can be produced, and the globule size distribution obtained is wide (25).

In making emulsions not only different apparatus but also emulsifying chambers of different size and outfits are used, which also influence the emulsification process. To minimize the influence of these variables during emulsification, an isothermal continuous recirculating system has been set up, where the flow velocity is controlled. This system is schematically represented in figure 5, and consists of a gear pump (1) connected to a reversible motor (2) and a revolution counter (3), an emulsifying chamber with in- and outlet (4), a heat exchanger (5) with a thermometer and plastic tubings, which complete the recirculating system. With this emulsification system, temperature control is much improved compared to batch-wise emulsification. It also provides an easier method for control of the emulsifying procedure; thus data obtained from this small scale equipment can more easily be scaled up for production requirements.



- 1 GEAR PUMP
- 2 REVERSIBLE MOTOR
- 3 REVOLUTION COUNTER
- 4 EMULSIFYING CHAMBER
- 5 HEAT EXCHANGER
- 6 THERMOMETER
- 7 PLASTIC TUBINGS

Fig. 5. A schematic representation of the isothermal recirculating, emulsification system (V).

The emulsifying part (4) has during the experiments been varied from emulsification with a turbomixer to sonication and to valve homogenization. Detailed description of each of the emulsifying apparatus used is given in paper V. A quantity of 50 grams was emulsified in all the experiments.

In order to compare the emulsifying efficiency of the apparatus, the power and energy consumption during emulsification have been measured and controlled. The net energy consumption, E , during emulsification in the recirculating system is given by:

$$E = P \cdot N_p \cdot t_p \quad (J)$$

P = net power input (W)

N_p = the average number of passes of an emulsion droplet through the recirculating system

t_p = the average residence time of an emulsion droplet during one passage (s)

The last parameter, t_p , has been set at a constant value of 12.5 ± 0.4 s during the experiments, giving a flow rate of the emulsion of 250 ml/min. The determination of the net power consumption, P , varies with the emulsifying apparatus used.

The measurement of the power generated by the turbomixer can be performed either by measuring the torque on the shaft and multiply it by revolution rate, or by measuring the heat dissipated during emulsification. The last alternative has been chosen as the torque is not easily measured because of the high rotation speed of the shaft. A 'calorimeter' to measure the heat dissipated during emulsification has been achieved by incorporating a heater into the continuous recirculating system. The system is described in paper V. It was determined, with an accuracy of ± 1 W with the calorimeter, that between 15 000 and 20 000 rpm the power transmitted to the emulsion by the turbomixer varied from 7 to 12 W.

The net power consumption of the valve homogenizer is equal to the pressure drop, Δp , times the flow rate, Q , of the emulsion, which gives:

$$P = \Delta p \cdot Q \quad (W)$$

Q can also be written as:

$$Q = V_s \cdot \frac{1}{t_v}$$

where V_s = stroke volume (4.6 ± 0.2 ml) and t_v = time valve is open during one stroke. Being a pneumatically driven, one piston homogenizer, the pressure drop varies with time during one stroke, and t_v varies with pressure drop. This has been taken into account in the calculations of the net power consumption. (For the details of the calculations, paper V is recommended). With only one piston working in the valve homogenizer, the transmission of power to the emulsion is made intermittently, hence during liquid flow the equation of the net energy input, E, is modified as follows:

$$E = P \cdot N_s \cdot t_v$$

N_s is the number of strokes and t_v is the time of valve lift.

The two most commonly used methods of measuring ultrasonic power are calorimetry and the radiation force method (30). Although the calorimetric method has a better theoretical basis, the radiation force method is more rapid and convenient to use. In this method one measures the radiation pressure force, F, exerted on a wall placed at a right angle in the path of an ultrasonic beam with a power, P. Thus we obtain:

$$F = \frac{P}{c}$$

where c is the velocity of sound in the medium in which the beam travels. The experimental set up consists of an open vessel containing emulsion, an electronic balance with an analog output coupled to a recorder, and the ultrasonic device, where the horn is dipped into the emulsion. If minor variations, due to the protein used as stabilizer, are involved, the net power consumption can be determined with an accuracy of ± 3 W.

C 2.2 Emulsion characterization

For every type of characterization of the emulsion formed, 30 g were stored for 24 hr at 20°C after formation.

Creaming stability. Creaming is the rise of droplets under the influence of gravity, which can be performed by single drops, aggregates or coalesced drops, as illustrated in figure 6. Various parameters can be chosen in evaluating creaming, such as depth of cream layer, percentage of the total fat that is collected in the cream layer, or the percentage of the total fat left in the aqueous lower phase. The percentage of water or of total solid in the aqueous lower phase can also be recorded. In this study the creaming stability (stability rating, SR) was determined as percentage change of fat in the aqueous lower phase after creaming. For details of the method the reader is referred to article I. The accuracy of the method is $\pm 1.5\%$ SR.

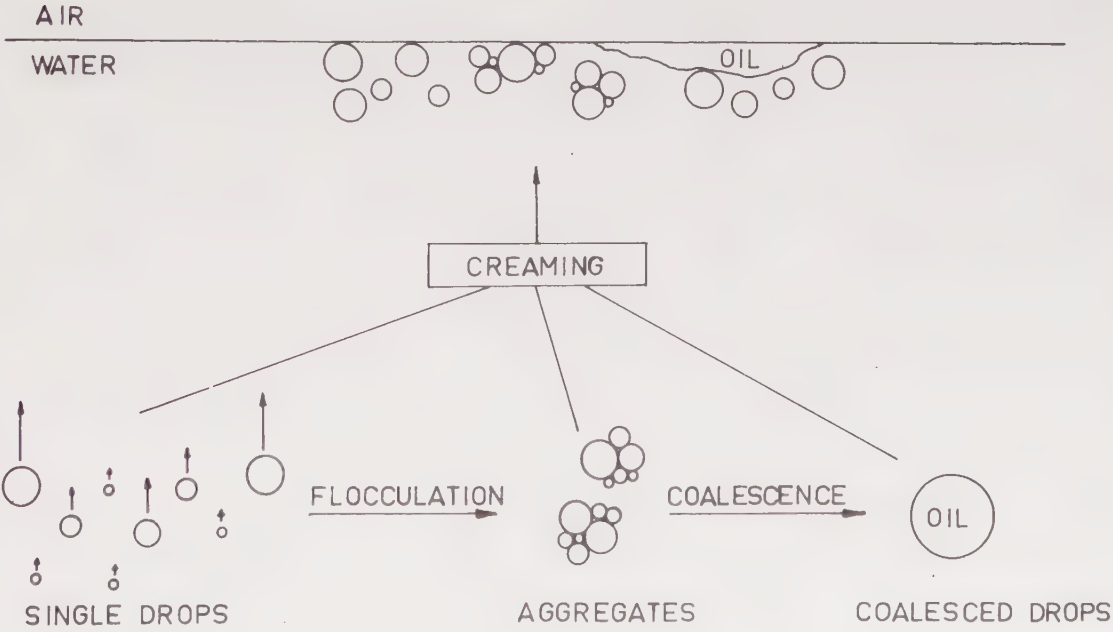


Fig. 6. A schematic representation of different mechanisms that cause creaming.

The size of the rising fat globules can be received from Stoke's law, but this equation must be used with care. Walstra and Oortwijn (31) have studied the applicability of the equation for milk up to a fat content of 8%. They concluded that creaming may differ considerably from the rate predicted by Stoke's law, particularly for milk of high fat content and for small globules, which is the case for the protein stabilized emulsions investigated in this study. Walstra and Oortwijn (31) stated as a first approximation, however, that viscosity and globule size are the most important variables in determining the creaming rate, and that increased polydispersity generally decreases the creaming rate.

Particle size distribution. Accurate determinations of the fat particle size distribution may cause problems, especially of the particles in the submicron range. Microscopic methods are applied most frequently, but the measurements are difficult and time consuming, and the results show poor reproducibility (32). Nevertheless, it is more or less a basic method, and the only one in which very small globules can be counted directly. For the very small particles fluorescence ($> 0.1 \mu\text{m}$) and electron microscopy are needed. Numerous methods employing sedimentation exist, either centrifugal or by gravity. Centrifugation in a density gradient may be suitable for evaluating size and number of very small globules (32). A sedimentometer combining centrifugal sedimentation and turbidimetric analysis has been used (33). The instrument follows the concentration change of globules in the suspending fluid at a fixed position in the centrifuge disk by recording the change in optical density. In the frequently used Coulter Counter method, the principle of measurements is based on the difference in electrical conductivity between emulsion particles and the diluent used. If all electrical errors that can be introduced during measurements and coincident passage of particles through the orifice are controlled, the method is accurate. The lower size range is, however, limited to a globule size of $0.7 \mu\text{m}$ diameter.

A spectroturbidimetric method was preferred in this study, as it offers a rapid, easy and reproducible method of analysis. Moreover, it can measure fat globules as small as $0.2 \mu\text{m}$ in diameter, but unfortunately not larger particles ($> 8 \mu$) and not

very broad size distribution (32). The method is fully described elsewhere (34, 35), and its application to the protein stabilized emulsions investigated in this study is described in article VII.

The average globule size obtained in the spectroturbidimetric method is the arithmetic mean of the surface-weighted distribution, denoted as d_{vs} . This mean is not so dependent on uncertainties in the small-globule end of the size distribution (25), and, moreover, it relates directly to the specific surface area, A , by the following formula:

$$A = \frac{6 \Phi}{d_{vs}} \quad \text{m}^2 \text{ fat/ml emulsion}$$

Φ is the volume fraction of the disperse phase.

The width of the size distribution is given as c_s , which is the relative standard deviation of the surface-weighted distribution. The reproducibility of the measurements is good, and the main error introduced by the method is probably due to the problems to fit the experimental data to the theoretical spectra, especially for very small d_{vs} (32).

Protein load determination. The amount of protein adsorbed per unit area of fat surface (protein load) can be obtained by subtracting the residual protein level in the bulk phase after emulsification from the original protein content, and dividing that value by the amount of interfacial area created. Methods to determine protein load are based on separation of the two dispersed phases, and then the protein concentration in the water phase (36) or on the oil phase (17) or in both (26) is measured. The interfacial area is derived from the particle size distribution according to the calculations described above.

For the emulsions examined in this study the following procedure was used. All the protein stabilized emulsions were centrifuged, and the lower water phase was compared in adsorbance with the original protein solution centrifuged at the same speed and of the same protein content. If the lower water enriched phase was more turbid than the original protein solution, this was taken as an indication of fat particles occurring in the water phase. In that case the water enriched phase was diluted 30 times, and

then filtered through a Millipore filter (GSPW, 0.22 μ). The determination of the protein content in the aqueous phase was made by analysing for nitrogen by the general Kjeldahl procedure with an accuracy of $\pm 0.5\%$. Probable errors that can be introduced by the method, such as sedimentation of protein, coalescence of fat globules during centrifugation, and protein retention in the filter have been estimated and are discussed in paper VII.

C 2.3

Emulsion_properties

Energy input. The creaming stabilities of differently emulsified emulsions stabilized by different proteins have been studied as a function of the net energy input, E (VI). It can generally be stated that although the same amount of energy has been transferred to the emulsion during emulsification, the creaming properties differ considerably as a function of the emulsifying equipment, the power generated or the number of passages through the emulsification system. An increase in energy input most commonly gives rise to better emulsions, in terms of creaming stabilities. If the power consumption is held constant and the number of passes is varied in order to obtain a variation in energy input, it is usually observed at the low power input of 10 W that the sonicated emulsions are superior in creaming stability to the valve homogenized ones at all energy levels, and these are in turn better than the emulsions produced in the turbomixer. It is usually more beneficial in terms of creaming stability to increase the power input at the expense of number of passes when emulsifying in the valve homogenizer than sonicating, in order to obtain the same energy consumption during emulsification.

Power input. The creaming stabilities (stability rating, SR) of the protein stabilized emulsions as a function of power consumption have been investigated in paper VI. Generally, an increase in power input improves the creaming stability of the emulsions up to a certain level, whereupon it levels off, except for valve homogenized soy protein (0.2 - 7) stabilized emulsions, which tend to be overprocessed in terms of creaming stability. Although almost all emulsions reach the plateau of high creaming stability, it is the rate of increase of the creaming stability

as a function of power input that differs between emulsions stabilized with different proteins. All the protein stabilized emulsions investigated are compared when valve homogenized at 10 passages in figure 7. It can be concluded from the figure that the addition of salt increases the creaming stability of caseinate stabilized emulsions, whereas the opposite is found for the two other proteins. An approximate order of the proteins in terms of creaming stability as a function of power input can be estimated from the figure, which is WPC (0 - 7) > WPC (0.2 - 7) $\approx$ soy protein (0 - 7) > caseinate (0.2 - 7) > soy protein (0.2 - 7) > caseinate (0 - 7).

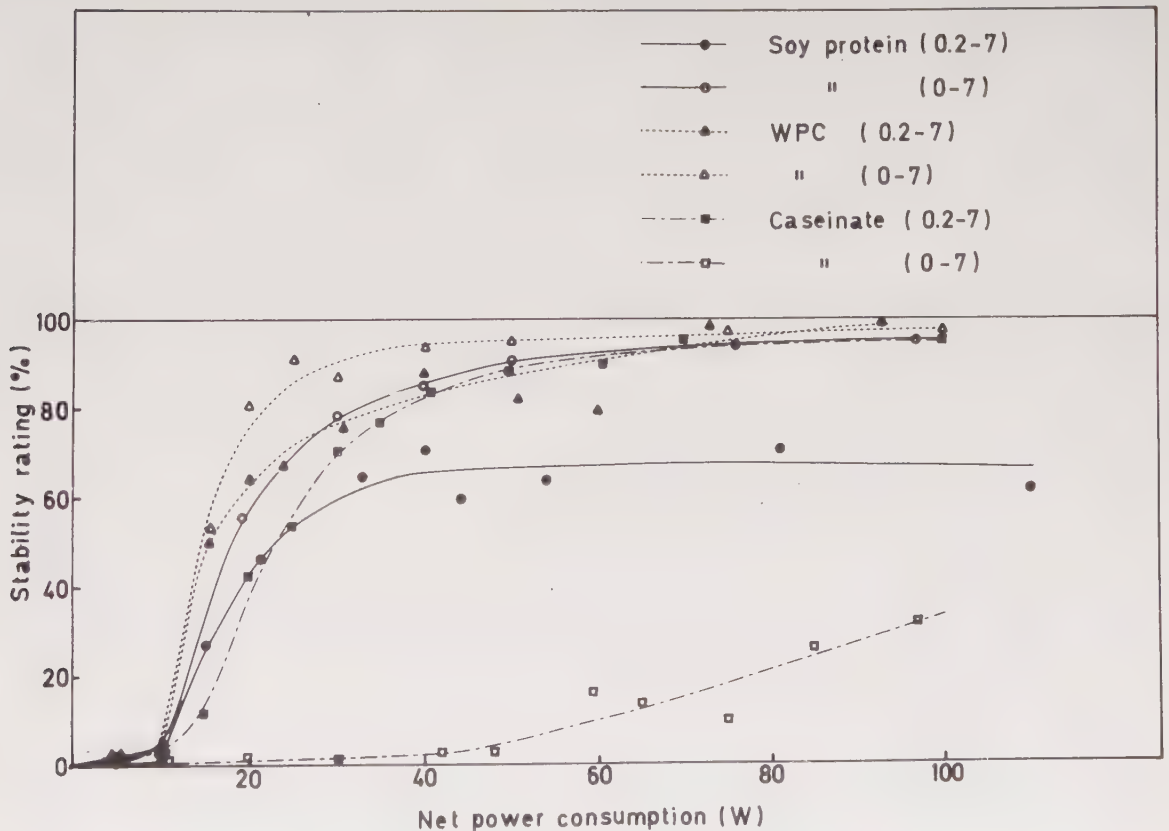


Fig. 7. The creaming stability (stability rating) of all the protein stabilized emulsions as a function of power input, on valve homogenization at 10 passages through the recirculating system (VI).

As suggested from figure 7 the improvement in creaming stability on increasing the power input is comparatively small for caseinate (0 - 7), which is confirmed in figure 8. A value as high as about 70 W is needed to reach the plateau value for 30 passages, whereas the other proteins only need 10 - 40 W to attain the same level of creaming stability. The difference in emulsifying behavior

of the ultrasonic device and the valve homogenizer is also very clear from figure 8. Sonication gives emulsions with a creaming stability that increases almost linearly with power consumption, whereas valve homogenization gives rise to emulsions in which the stability rating increases more abruptly with power input. This difference in creaming behavior can be due to the fact that sonication gives emulsions with very small particles but a wide spread in globule size compared to valve homogenization (25).

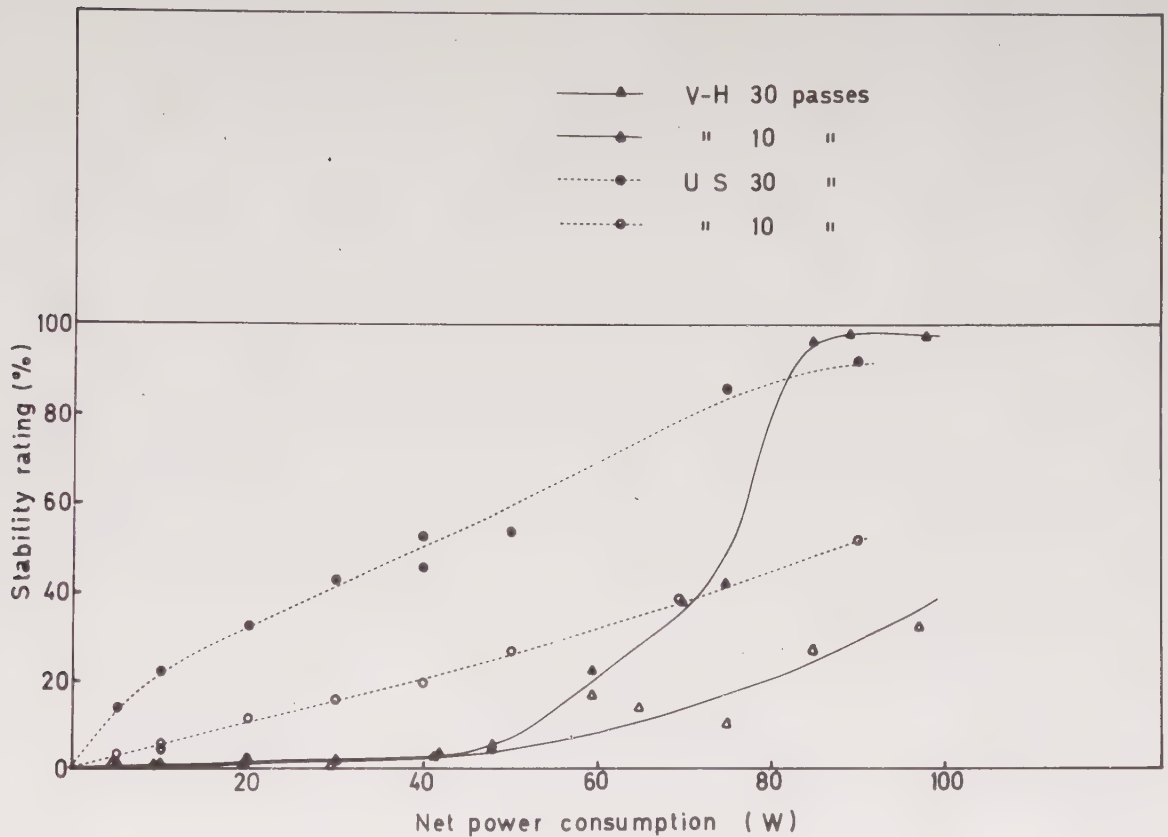


Fig. 8. Creaming stability (stability rating) of caseinate (0 - 7) stabilized emulsions as a function of power consumption on sonication (U-S) and valve homogenization (V-H) at 10 and 30 passages, respectively (VI).

The fat surface area and the width of the size distribution, c_s , respectively, are plotted in figures 9 and 10 against power input during valve homogenization at 10 passes (VII). The almost linear curves up to 40 W in figure 9 coincide within the limits of error for the WPC and caseinate stabilized emulsions. The divergence between the protein stabilized emulsions observed at higher power inputs than 40 W, give rise to emulsions of smallest globule size and largest surface area for those stabilized by soy protein (0 - 7), followed by caseinate (0.2 - 7), WPC (0 - 7) and

caseinate (0 - 7). The surface area of the three last mentioned emulsions more or less continue the linear dependence on power input. The curves obtained for WPC (0.2 - 7) and soy protein (0.2 - 7) adopt a more sigmoidal behavior, where the latter gives emulsions of largest globules and thereby the smallest fat surface area.

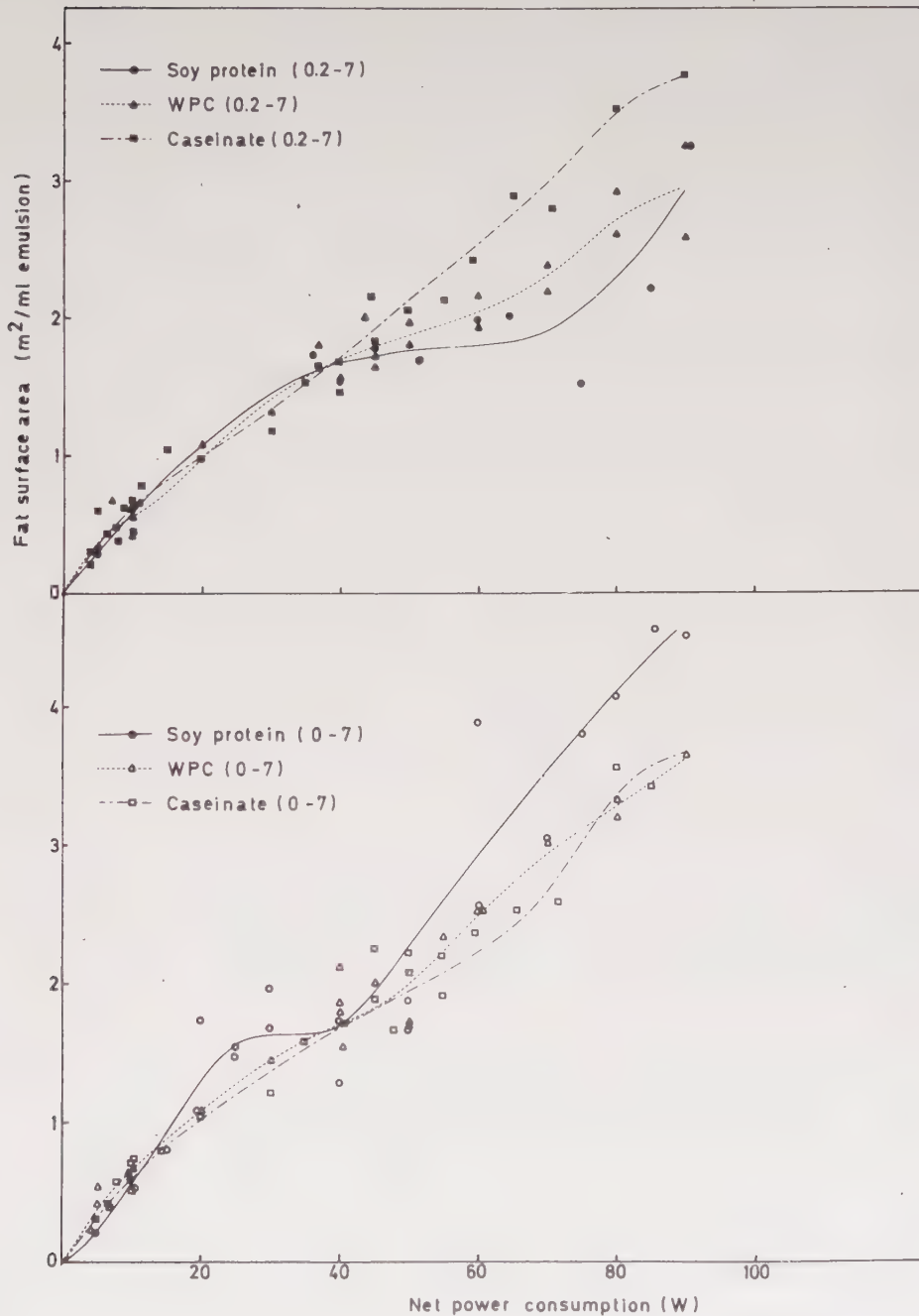


Fig. 9. Fat surface area of protein stabilized emulsions as a function of net power consumption after emulsification with a valve homogenizer using 10 passes through the recirculating system (VII).

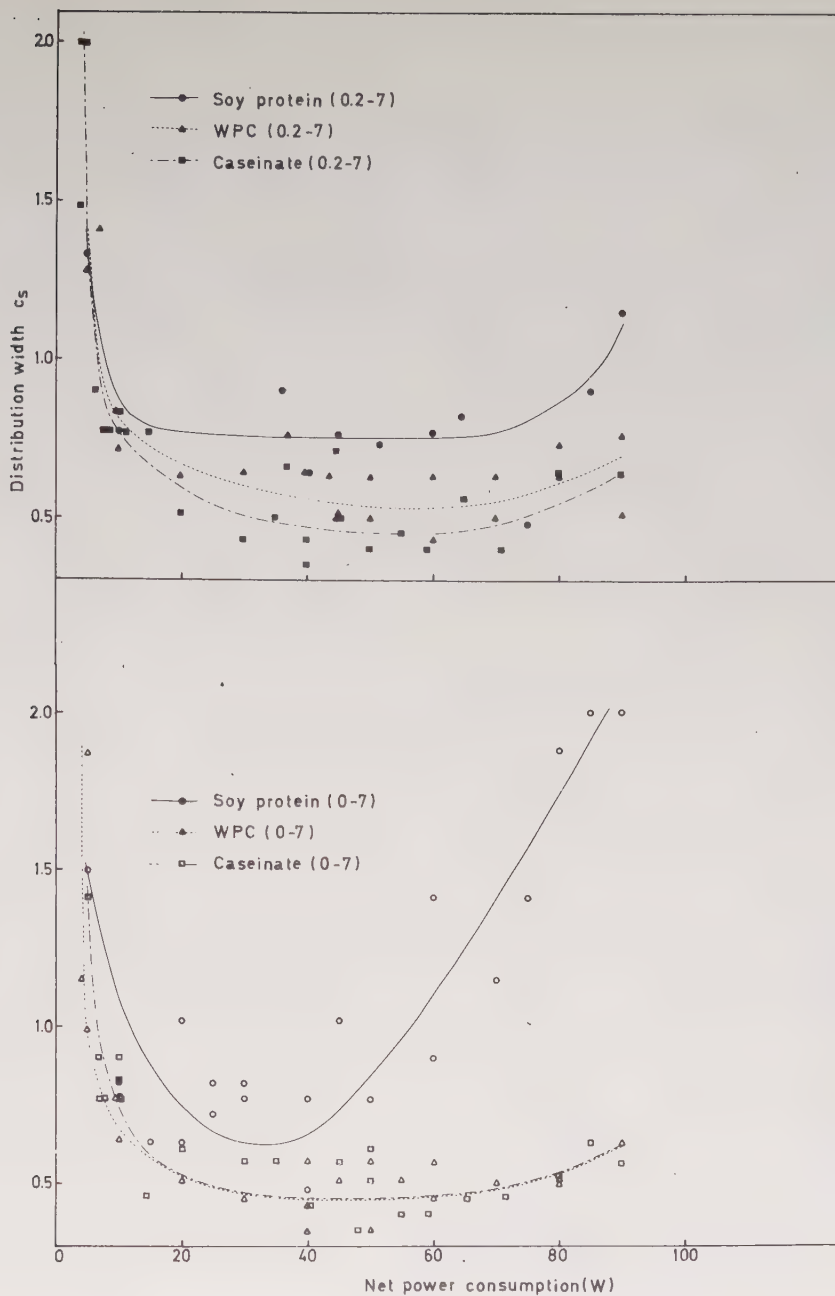


Fig. 10. Distribution width, c_s , of the protein stabilized emulsions as a function of net power consumption, on emulsification with a valve homogenizer using 10 passes through the recirculating system (VII).

In figure 10 it can be seen that both soy protein (0.2 - 7) and soy protein (0 - 7) give rise to emulsions with larger spread than the other proteins, which is most pronounced at higher levels of power input. Every curve in figure 10 shows a minimum in distribution width, c_s , in the medium range of power input, whereafter c_s increases again at the highest power consumptions. This minimum in c_s is rather flat ranging from 30 to 60 W except for the soy protein (0 - 7) stabilized emulsions, where the minimum in c_s is between 20 and 40 W, and the rise

in c_s at higher power inputs than 40 W is steep reaching a value of c_s as high as 2.0 at 85 - 90 W.

In figure 11 the amount of protein adsorbed in mg per m^2 fat surface area is plotted against fat surface area created by augmenting the power input in accordance with figure 10 (VII). Figure 11 gives clear evidence that not only the size of the fat globules and thereby the fat surface area, but also the amount of interfacial film is strongly affected by the way the emulsions are made, which in turn influences the properties of the emulsions formed.

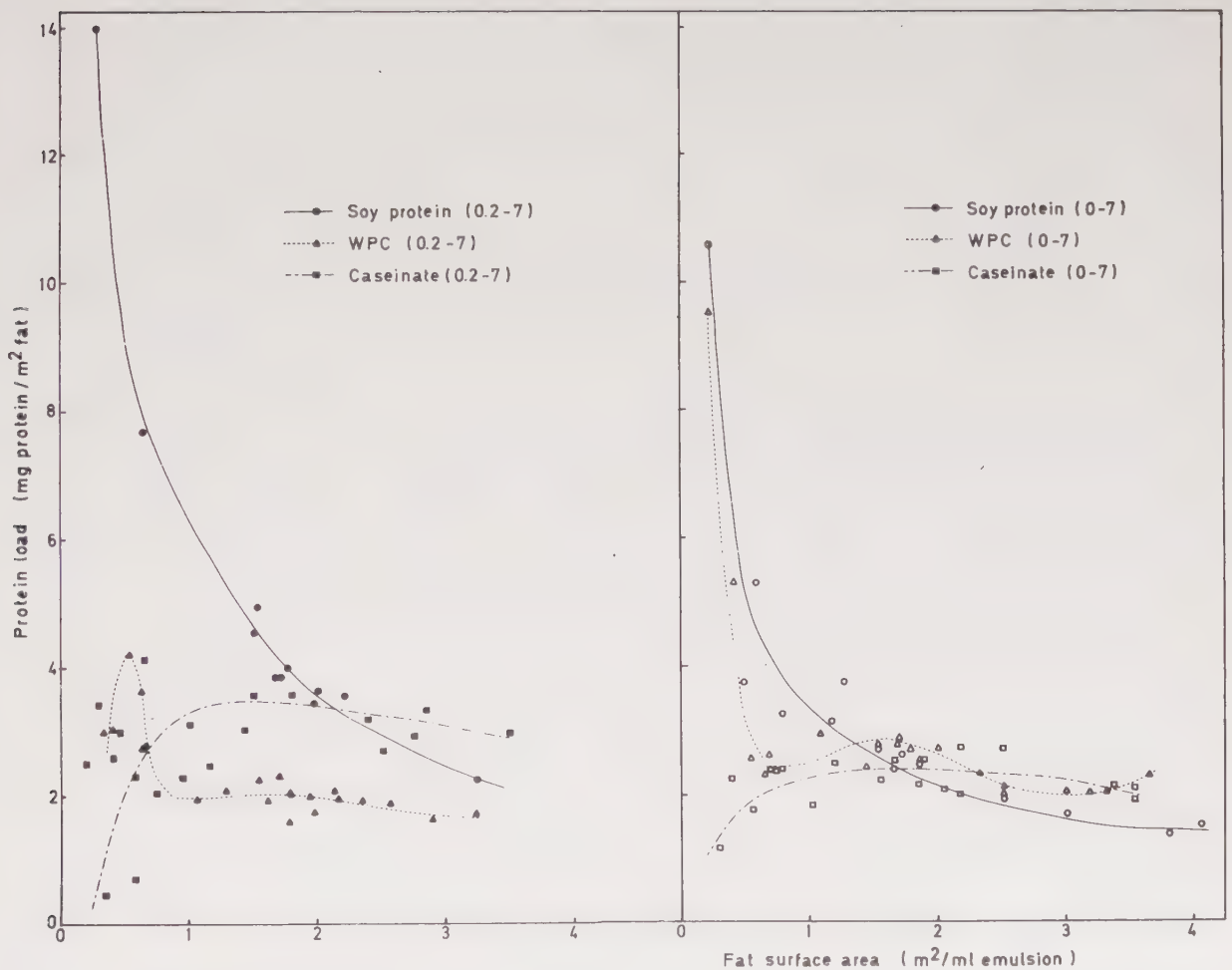


Fig. 11. Protein load of the protein stabilized emulsions as a function of the fat surface area created during variation of power supply at a constant number of passes (10) (VII).

The most striking feature to be observed in figure 11 is the very different behavior shown by the caseinates as compared to the soy proteins. The emulsions stabilized by the latter have

a very high protein load at small surface areas, which diminishes almost exponentially as the surface area expands. The caseinates, though, have a low value of protein load for emulsions of small surface area, but it grows as a function of the surface area to a maximum at about $1.5 \text{ m}^2/\text{ml}$ emulsion, whereafter it decreases slightly. For both these proteins the addition of salt results in more protein adsorbed at the interface for all the surface areas investigated. In this connection it can be interesting to compare the values obtained by Graham (17) for the maximum amount of irreversibly adsorbed β -casein ($\text{pH} = 7$; ionic strength = 0.1) at the air-water interface, which comprises the primary layer of molecules. His data of $2.7 \text{ mg}/\text{m}^2$ is in between those obtained for caseinate ($0.2 - 7$) and caseinate ($0 - 7$) at surface areas larger than $1.0 \text{ m}^2/\text{ml}$, which suggest that the protein coverage under these conditions is composed of a monolayer, being more densely packed at the higher ionic strength. As the association and aggregation of the 7S and 11S globulins of the soy proteins are favoured at $0.1 - 0.2 \text{ M NaCl}$, it may explain the higher protein load observed for soy protein ($0.2 - 7$) when compared to soy protein ($0 - 7$).

The whey proteins show behavior somewhat intermediate to the two other proteins. The WPC ($0.2 - 7$) stabilized emulsions have the lowest protein load ($\approx 2 \text{ mg}/\text{m}^2$) at fat surface areas between 1.0 and $2.0 \text{ m}^2/\text{ml}$, whereas at larger surface areas the soy protein ($0 - 7$) stabilized emulsions have as low values as those stabilized with WPC ($0.2 - 7$). It is interesting to note that an increase in ionic strength to 0.2 M NaCl does not increase the amount of protein adsorbed in the case of the whey proteins. In fact the opposite is observed, in contrast to the behavior of the other two proteins.

In order to correlate the creaming properties with the fat particle size and the properties of the protein film around the fat droplets, figures 7, 9, 10 and 11 can be compared. Moreover, the viscosity of the emulsions when valve homogenized at 40 W and 10 passes, has been evaluated, as it is known that viscosity strongly influences the creaming properties of emulsions (31).

In figure 12 viscosity measured with Contraves Rheomat 30 as a function of shear rate is given for all the protein stabilized emulsions except for the soy protein (0.2 - 7) stabilized emulsions. The latter emulsions were better evaluated in the low shear range, because both the high viscosity and elasticity exhibited by the soy protein (0.2 - 7) stabilized emulsions were irreversibly lost at a shear rate below 1 s^{-1} . No significant elasticity or irreversible tixotropy was observed for the other protein stabilized emulsions. The irreversible breakdown of viscosity of soy protein (0.2 - 7) stabilized emulsions, where the fat particles were highly aggregated as revealed by microscopy, indicates the formation of bridges between the fat droplets; the same mechanism which has been proposed to explain the viscosity rise in homogenized cream (36). The bridging concept means that a protein already adsorbed on a fat particle can extend far out in the continuous medium and reach another nude fat globule and adsorb thereon. Van Ogden *et al.* (36) have evaluated the factors that favour bridge formation in homogenized cream, which are high pressure drop, large fat fraction and large surfactant molecules. This seems to be in accordance with the observations made in this study, *i.e.* significant viscosity rise during homogenization was only observed for those emulsions stabilized with the highly aggregated soy protein (0.2 - 7), and furthermore this increase in viscosity was more evident at higher pressure drops. The difference in creaming behavior by the protein stabilized emulsions in figure 7 is not simply correlated to any of the emulsion properties measured and presented in figures 9, 10, 11 and 12.

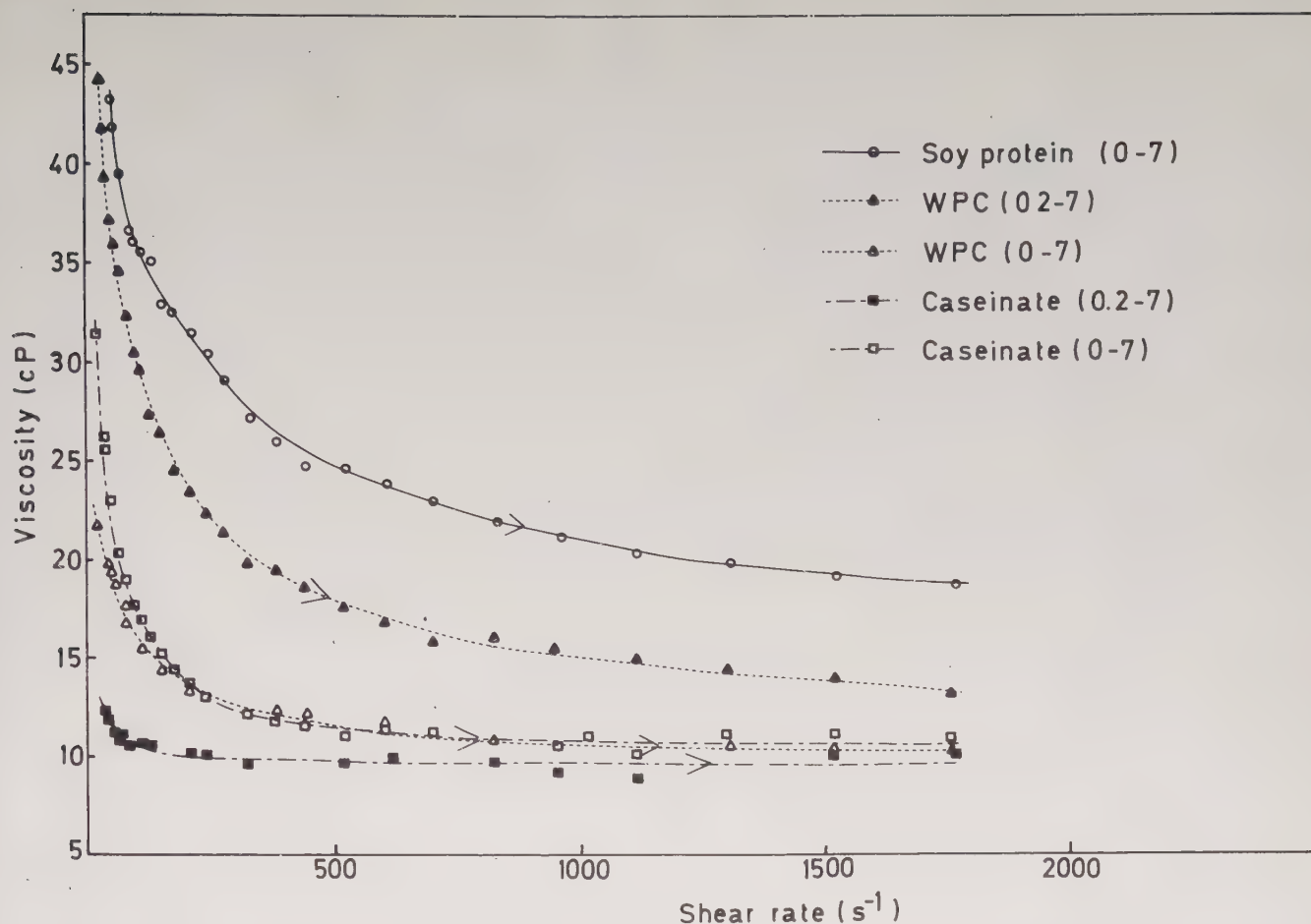


Fig. 12. The viscosity of all the protein stabilized emulsions except for those stabilized with soy protein (0.2 - 7) as a function of shear rate. The emulsions were made in a valve homogenizer at a power supply of 40 W and with 10 passes through the recirculating system.

Number of passes. The creaming stabilities of the protein stabilized emulsions as a function of number of passes at constant levels of 10 and 40 W power input have been investigated in paper VI. Some typical features observed for all the protein stabilized emulsions can be reported. At the lower power input the ultrasonic equipment gives emulsions in which the rate of growth of creaming stability is very high compared to the valve homogenized emulsions. The emulsions obtained at 40 W are always superior in creaming stability to those obtained at 10 W for the same number of passes, and an increase of the number of passes usually improves the creaming stability of the emulsions formed. Both with the variation of power and passes it has emerged from the findings that the valve homogenizer is a good choice of emulsifying equipment for the preparation of creaming stable WPC-stabilized emulsions. This is also verified by figure 13, where all the proteins can be compared in giving emulsions of good creaming stability on valve homogenization at 40 W. The order of the protein stabilized emulsions with respect to creaming stability is similar to the

one observed in figure 7, where the power input has been varied.

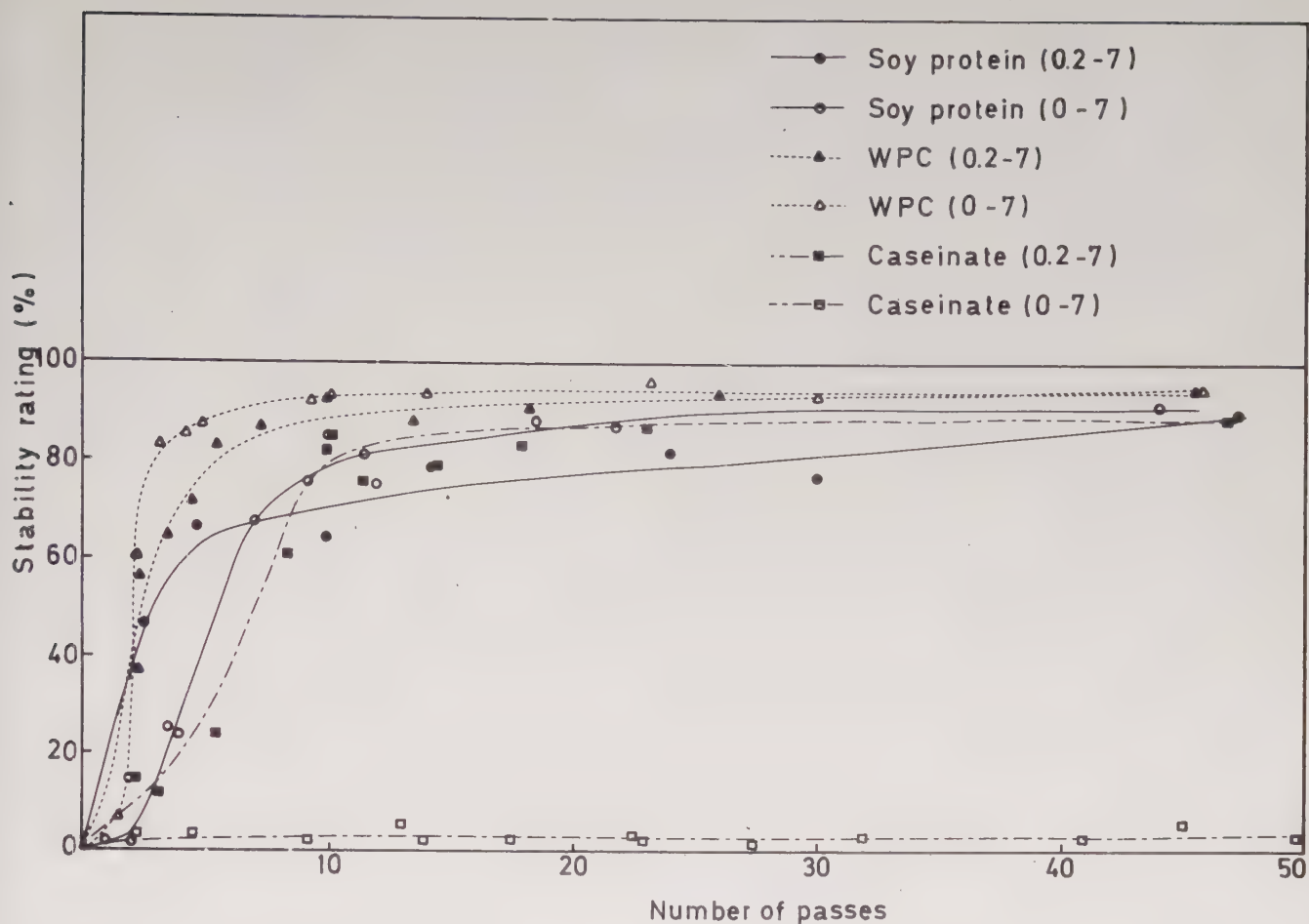


Fig. 13. Creaming stability (stability rating) of all the protein stabilized emulsions as a function of number of passes, on valve homogenization at a power supply of 40 W.

Figure 14 (VII) shows the fat surface area of all the protein stabilized emulsions, when valve homogenized as a function of the number of passes at a power consumption of 40 W. For the WPC (0.2 - 7), caseinate (0.2 - 7) and caseinate (0 - 7) stabilized emulsions there is a weak and almost similar dependence on the number of passes. The WPC (0 - 7) stabilized emulsions are, however, more sensitive to the number of passes. The soy protein stabilized emulsions behave somewhat differently, both having a rather wide scatter in results, which is especially pronounced for soy protein (0.2 - 7).

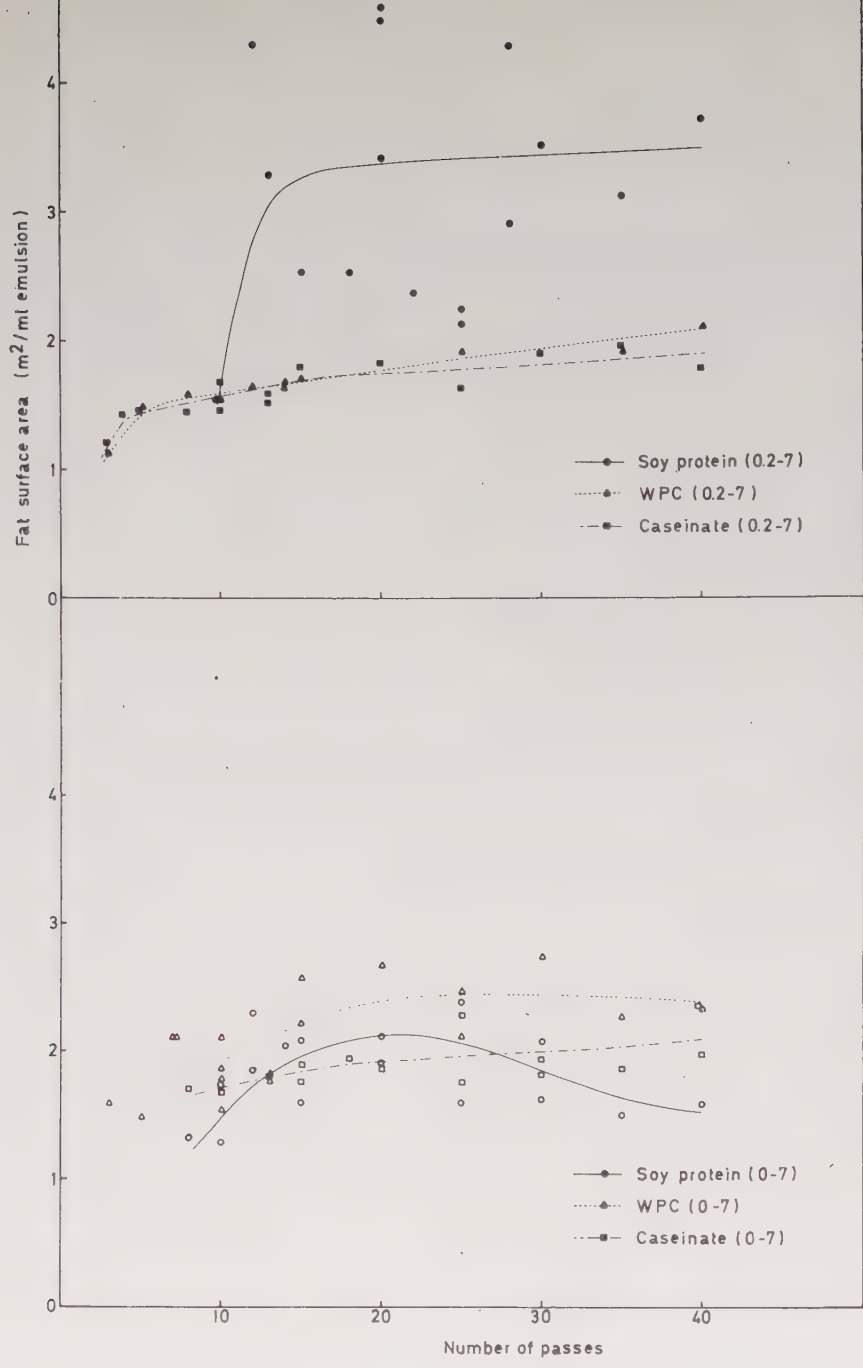


Fig. 14. Fat surface area of protein stabilized emulsions as a function of number of passes after emulsification with a valve homogenizer at constant power supply of 40 W (VII).

In figure 15 (VII) distribution width, c_s , as a function of number of passes is plotted at a constant power supply of 40 W, when the emulsions are valve homogenized. For the WPC and caseinate stabilized emulsions the size distribution becomes narrower for increased number of passes, whereas soy protein (0.2 - 7) stabilized emulsions get larger width. In comparison with figure 10 it can be seen that the narrowest size distribution ($c_s \approx 0.3$) can be obtained by emulsifying at a high number of passes.

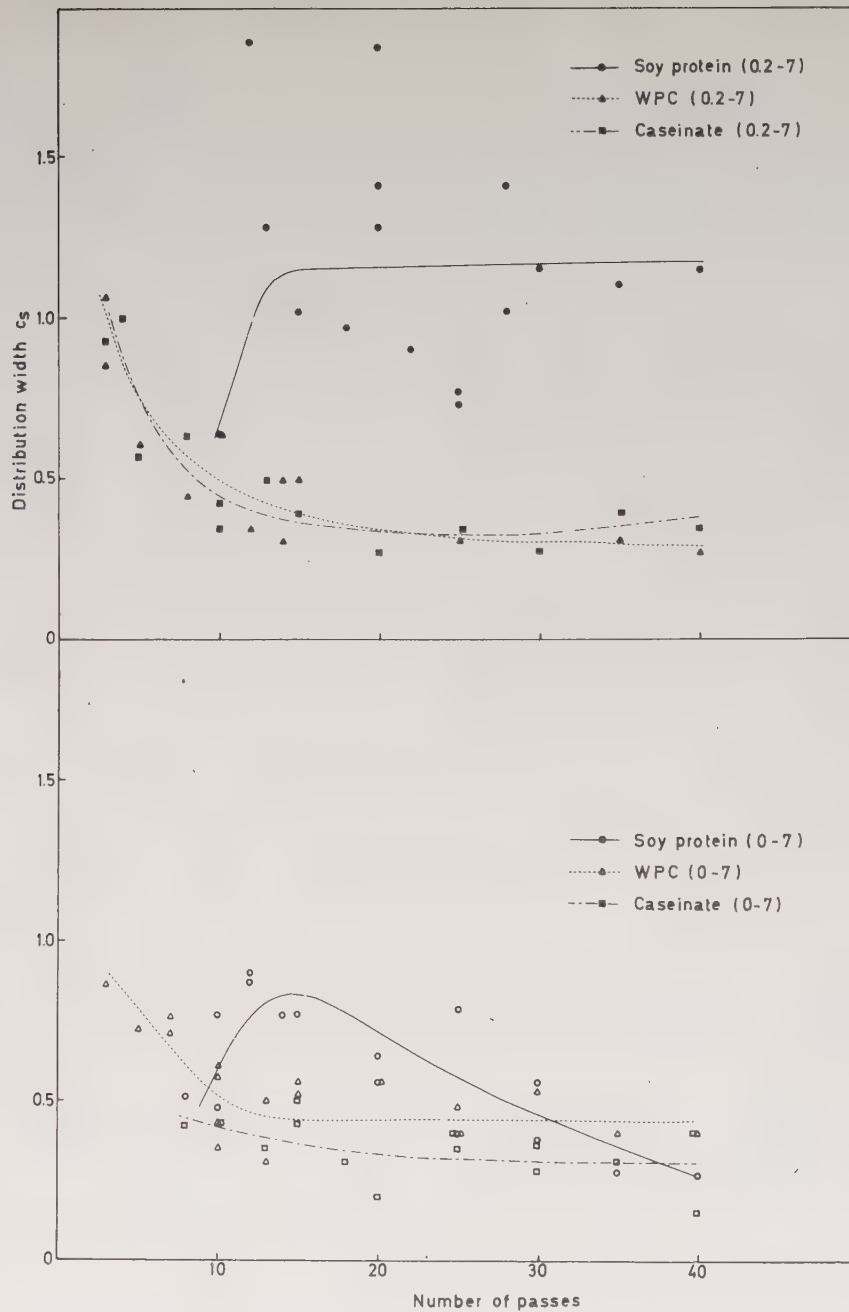


Fig. 15. Distribution width, c_s , of the protein stabilized emulsions as a function of number of passes, on emulsification with a valve homogenizer at a constant power supply of 40 W (VII).

In figure 16 (VII) the same type of curves have been constructed as those in figure 11, but in this case the fat surface area has been created as a function of number of passes in accordance with figure 14. For the soy protein (0.2 - 7) stabilized emulsions and for the WPC stabilized emulsions, the curves in figure 16 agree well and with minor deviations, respectively, with those in figure 11. This shows that the protein load will be the same for the same fat surface area, irrespective of how this area has been created. The both caseinates show a stronger dependence in protein load on the fat surface area, and there is a little

higher protein coverage at the same fat surface area for the soy protein (0 - 7) stabilized emulsions, when the fat surface areas are generated by variation in number of passes instead of power supply.

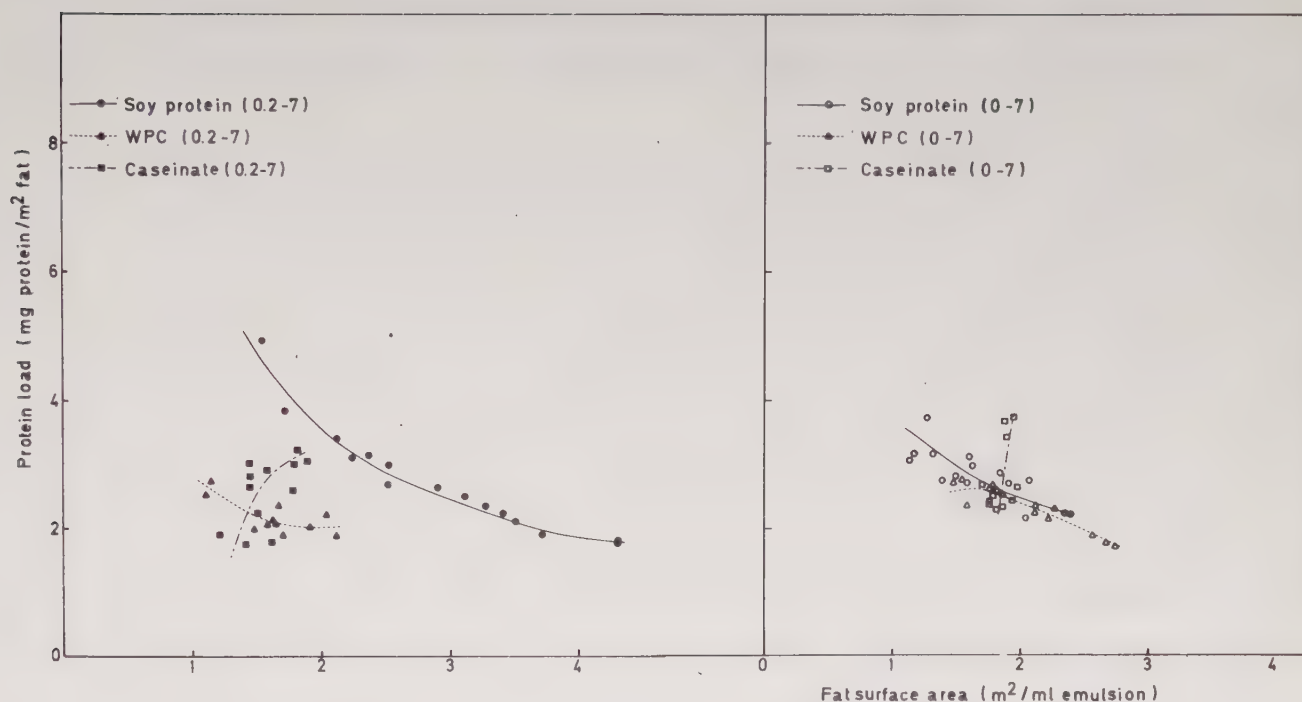


Fig. 16. Protein load of the protein stabilized emulsions as a function of the fat surface area created during variation of number of passes at a constant power supply of 40 W (VII).

As in the case of variation of power supply, no clear relation can be found by comparing figures 13 - 16 between the creaming stabilities of the emulsions and the other investigated properties. The large difference in creaming stability observed for the caseinate stabilized emulsions with and without salt occurs both in figures 7 and 13. It cannot be explained by a difference in particle size or spread in globule size. There is, though, according to figure 12, a slight difference in viscosity, where caseinate (0 - 7) is more viscous than caseinate (0.2 - 7), indicating a more flocculated emulsion for the former. The assumed flocculation of the caseinate (0 - 7) stabilized emulsions is not observed in the microscope, and the eventually occurring floccules seem to be redispersed on dilution. The phenomena observed suggest a reversible aggregation in the shallow secondary minimum according to the DLVO-theory (37).

GENERAL DISCUSSION

Emulsification is a dynamic process involving disruption and recombination or coalescence of fat globules. Thus, the particle size distribution and other properties of the emulsion formed will be governed by the detailed conditions of the balance between disruption and coalescence during emulsification.

The time for globule break-up ranges from 9.0 to 2.8 μ s in the power range covered for the valve homogenizer used in this study (V). Because this time of disruption is very short, the degree of coalescence during emulsification can be considered to be negligible. After exit from the valve of the dispersed fat globules, coalescence, however, grows in importance during the 12.5 s it takes on average for one droplet to pass the recirculating system once. The probability for the newly created droplets not to coalesce depends on the time available for the interfaces to be covered by proteins. This time is determined by the probability of encounter between droplets. It is also determined by the rate of supply of polymer molecules from the bulk, and by the rate of spreading of already adsorbed molecules (38, 18), because the rate of these mechanisms influences the persistence in the surface tension gradients. If the interface is covered mainly by protein molecules emerging from the bulk, the streaming arising in the continuous phase separates the droplets, which will prolong the time available for the interface to be covered by proteins. In addition to this supply mechanism of polymer molecules or segments at the interface, the structure of the final film at the interface also influences the final particle size distribution after emulsification.

The probability of collision between two droplets is mainly dependent on the number of droplets per unit volume. The rate of supply of protein molecules from the bulk depends on the number of collisions of protein molecules with a fat particle per unit volume and time, and on the probability of the protein molecule to adsorb when colliding with the interface. The number of collisions of the protein molecules is governed by molecular or eddy diffusion or both depending on the flow conditions during emulsification. According to calculations by van Ogden et al. (36) the adsorption of surfactants on to the

fat globule interface during valve homogenization is expected to be turbulence controlled. By making some simplifications they derived an equation describing the conditions during adsorption of proteins in turbulent flow, showing that larger protein particles are adsorbed preferentially on the fat globules compared to the smaller ones, especially for globules of comparable size. These conditions are assumed to reign just after the valve exit, but as the kinetic energy will diminish continuously further away from the valve, molecular diffusion becomes more important. The rate of spreading of an already adsorbed protein molecule will depend on its flexibility on the surface and on the surface pressure it has to expand against. With regard to the influence of the structure of the final protein film on coalescence, Graham (17) has found that coalescence will be reduced, when the protein layers are thick, highly hydrated and charged.

In order to bring about a correlation between the results obtained in the interfacial and the emulsification studies, the amount of protein available in the bulk before adsorption in relation to the interface created has been set equal in the both studies. This calculation procedure gave rise to the relation visible in figure 17, where the interfacial area created, when making emulsions, is plotted against the initial subphase concentration (wt. %) in the interfacial tension study. It can be seen in the figure that the approximate upper limit of the interfacial area of the emulsions of $4.0 \text{ m}^2/\text{ml}$ corresponds to an initial subphase concentration of $5 \cdot 10^{-3}$ wt. % in the interfacial tension measurements. According to the interfacial tension results presented in figure 2, the proteins studied can roughly be divided into two groups in this subphase concentration range; the first group consisting of the WPC and the caseinates covering the interface relatively rapidly, and the second group consisting of the soy proteins covering the interface slowly. It might be argued against these comparisons of the interfacial and the emulsification studies, that the former has been done at the air-water interface and the latter at the oil-water interface. Graham (17) has shown, however, that the adsorption for β -casein and BSA at the air-water interface are similar to those obtained at the oil-water interface.

Some interfacial tension measurements at the soybean oil-water interface of the present proteins also indicate the same relationship between the proteins as at the air-water interface.

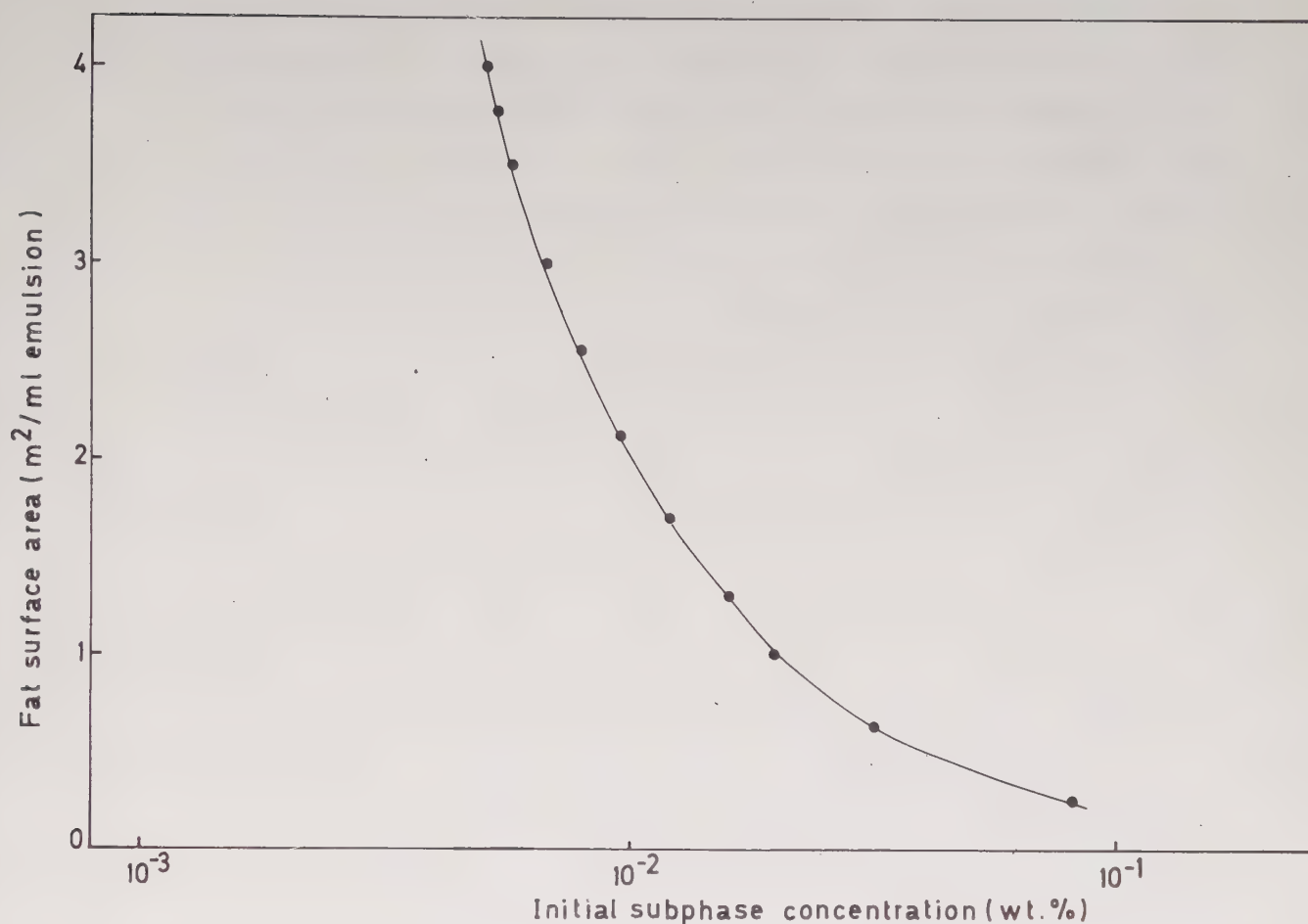


Fig. 17. The correspondence between the interfacial area of an emulsion made with an initial protein content of 2.5% (w/w) in the continuous phase and the initial subphase concentration during the interfacial tension measurements, if the amount of protein available in the bulk before adsorption in relation to the interface is set equal (VII).

The percentage protein adsorbed from bulk is plotted as a function of fat surface area created by increasing the power input during valve homogenization in figure 18. There is a higher percentage adsorption by the soy proteins at small surface areas and a lower percentage adsorption at the larger surface areas compared to the caseinates. To cover the interface with protein at an enlarged surface area the caseinate, especially in 0.2 M NaCl, predominantly supply protein from the bulk. The soy proteins, though, to a lesser extent, supply protein from the bulk especially at larger areas than $2.0 \text{ m}^2/\text{ml}$, where percentage adsorbed is more or less linear as a function of fat

surface area. This indicates that at these surface areas the newly created interface is mostly covered by spreading or unfolding of the already adsorbed soy protein molecules at the interface. For both proteins, salt addition induces more protein to be adsorbed at the interface for all the surface areas investigated. This emulsifying behavior of the two proteins should also be expected from the interfacial tension measurements. The high protein coverage of the soy proteins at small surface areas is in accordance with the soy proteins migrating to the interface by the gross associated complex, whereas the low percentage adsorbed for the caseinates at these surface areas support the assumption that casein monomers are first adsorbed at the interface. The pronounced supply from bulk even at large surface areas for the caseinates, especially for caseinate (0.2 - 7), could be deduced from the interfacial tension measurements by the fact that diffusion is the rate-controlling step to a high Π . The increased adsorption of the caseinate when salt is added could also be expected from the interfacial tension results, giving a higher diffusion rate and a lower adsorption barrier for caseinate (0.2 - 7) compared to caseinate (0 - 7). The interfacial tension results showed that the soy proteins spread relatively easy at the interface, especially in 0.2 M NaCl solution, and this is mainly the way the soy proteins cover the enlarged interface during emulsification, as suggested from figure 18.

The emulsifying behavior of the whey proteins is not as easily correlated with the interfacial behavior as that of the other proteins. The interfacial data showed that WPC (0 - 7) spreads relatively easy on the interface in relation to WPC (0.2 - 7), but in figure 18 the supply of molecules from bulk by WPC (0 - 7) exceeds that of WPC (0.2 - 7). This might be explained if the unfolding of the WPC (0 - 7) molecules gives rise to hydrophobic groups exposed to the bulk. This induces further adsorption of protein from bulk and, hence, multilayer formation. The peculiar increase in percentage adsorption of WPC (0 - 7) molecules at large surface areas, which follows on a preceding step of spreading, is a further indication of this phenomenon.

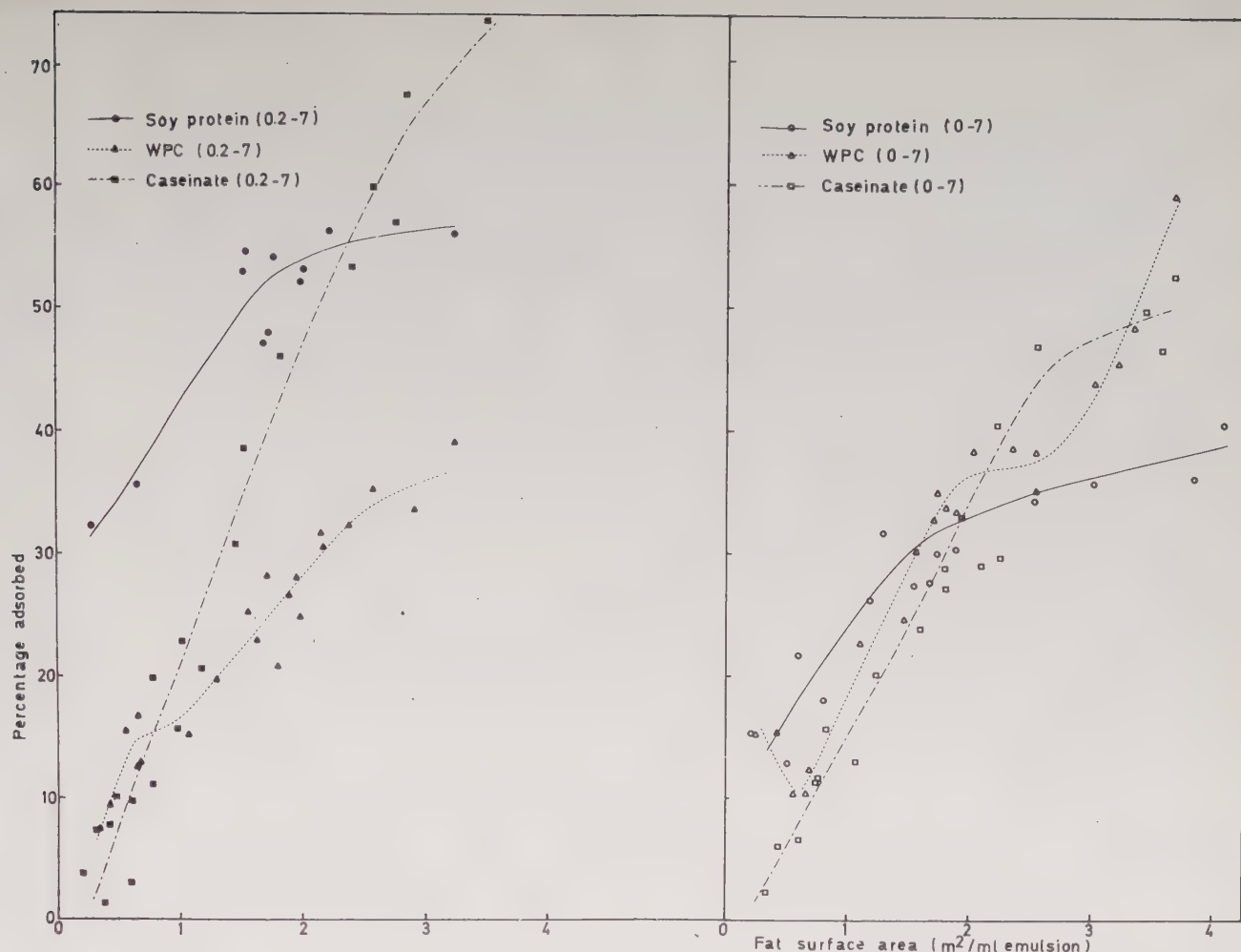


Fig. 18. Percentage adsorbed protein from the bulk to the interface of the protein stabilized emulsions as a function of the fat surface area created during variation of power supply in a valve homogenizer using a constant number of passes (10) (VII).

The ability of the proteins to give emulsions of small fat particles as a function of power input can be seen in figure 9. There is no significant difference in particle size distribution for the WPC and caseinate stabilized up to 40 W, where the surface area (1.75 m²/ml) approximately corresponds to a subphase concentration of 10⁻² wt. % in the interfacial studies. The interfacial tension measurements indicated that at concentrations $\geq 10^{-2}$ wt. % the diffusion of WPC and caseinate to the interface is rapid and rather similar. From figure 18 it can be seen that the amount of protein supplied from the bulk is rather similar at small surface areas, but diverges for enlarged surface areas; being most pronounced for caseinate (0.2 - 7) followed by caseinate (0 - 7) and WPC (0 - 7) at almost the same level and finally WPC (0.2 - 7) having the lowest level adsorbed. This order is the same as observed in figure 9 for the fat surface area created. Evidently, both the amount supplied from bulk and the

rate of adsorption influence the final particle size distribution, so that it is advantageous in order to get a large surface area to cover the newly created interface slowly by adsorption from bulk. This is consistent with the mechanism, where the supply from bulk induces streaming of the continuous phase and thereby separation of the newly formed droplets. This mechanism could also account for the difference in surface area obtained for the two soy proteins. The interfacial tension measurements revealed that diffusion to the interface is accomplished at a much slower rate for soy protein (0 - 7) than for soy protein (0.2 - 7), and that the tendency to spread of soy protein (0 - 7) is less at the interface. Due to this observed interfacial behavior of the soy protein (0 - 7), the surface tension gradients occurring during emulsification persist over longer periods of time. The diffusion of the soy protein (0.2 - 7) is also slow compared to the two other proteins, but it spreads most easily of all the proteins studied, and the relaxation of the surface tension gradient in this case gives rise to much less separation between the droplets. As a probable result of this the soy protein (0.2 - 7) stabilized emulsions have the smallest fat surface areas.

In repeated passes at constant supply of power, the resistance of the protein covered fat globules to withstand repeated homogenization is of importance. Surface renewal on repeated homogenization will depend on the mechanisms that govern globule disruption. Walstra (39) has shown that Kolmagoroff's theory of isotropic turbulence can be applied to the mechanism of globule disruption when valve homogenizing milk. Kolmagoroff's theory only considers the interfacial tension decay, γ , caused by the adsorption of the emulsifier, and the fat particle size (d_{VS}), but not the influence of the already adsorbed protein molecules on the resistance of the fat globule to withstand further disruption. Taking this into account the local fluctuating eddy pressure, Δp , in the turbulent motion during emulsification, must overcome $(4 \gamma / d_{VS} + \Pi / \delta)$ in order to disrupt the globule. Π is the surface pressure caused by the already adsorbed molecules, and δ is roughly the thickness of the surface film.

When comparing the interfacial data with the emulsifying properties of WPC and caseinate, it seems to be the rate of building up the surface pressure that determines the resistance to further homogenization. The interfacial decay is in the current concentration range most slowly for WPC (0 - 7), followed by caseinate (0 - 7), WPC (0.2 - 7) and caseinate (0.2 - 7), which is in accordance with the relative magnitude of the fat surface areas shown in figure 14. Interfacial tension decay lowers γ but gives at the same time a rise in Π , where the former variable facilitates and the latter opposes disruption. It can, though, be concluded from the results in this study that the effect of the interfacial tension decay by proteins is an enhanced resistance to disruption at repeated homogenization due to the build up of surface pressure, Π .

The results obtained for the soy proteins are very scattered in figures 14 and 15, especially for soy protein (0.2 - 7), which indicate a high probability for the globules to undergo further disruption in a randomized way. This can be due to the comparatively slow coverage of the interface by the soy proteins.

SUMMARY

The aim of this thesis has been to find out the emulsifying properties of food proteins, represented by a soy protein isolate, a whey protein concentrate (WPC) and a sodium caseinate, in order to gain knowledge of how proteins can be supplemented into already existing foods, and how they can replace traditional foods. The thesis can be divided into two parts, the first one dealing with interfacial studies of the proteins, and the second one involving emulsification studies. In the general discussion the results from the interfacial and emulsifying behavior of the proteins have been combined to give a more general view of proteins as emulsifiers.

In the interfacial tension studies a new construction of an apparatus based on the drop volume principle has been used, and procedures for time-independent as well as time-dependent interfacial tensions have been worked out. The kinetics of protein adsorption at the interface has been recorded, and it has been analysed in terms of distinguishable rate-determining steps. From this analysis the following characteristics concerning the interfacial behavior of the three protein systems have been deduced.

The soy proteins diffuse slowly to the interface compared to the other proteins, which may be due to the large particle size of the association complex of the soy proteins. For the soy proteins diffusion is slower in distilled water than in 0.2 M NaCl solution and spreading at the interface is most easily performed in 0.2 M NaCl solution. The whey proteins diffuse quickly to the interface, which is in accordance with their aqueous association; mainly small molecular complexes. WPC in distilled water diffuses more slowly but spreads more easily at the interface than in 0.2 M NaCl solution. Although the caseinate has a complex quaternary structure, like the soy proteins, it has a very different interfacial behavior. The migration to the interface of the caseinates is proposed to be performed mainly by the free casein molecules, which are in equilibrium with those in the submicelles. The diffusion controlled occupation of the interface is most evident for the caseinates, especially in 0.2 M NaCl solution.

In the emulsification studies, preparation of emulsions of 40% soybean oil by weight has been performed in an isothermal, recirculating system. Power and energy consumption during emulsification have been measured and controlled. The emulsifying part in the recirculating system has been varied from emulsification with a turbomixer to sonication and to valve homogenization. The emulsions formed were characterized in terms of creaming stability, fat particle size distribution and amount of protein adsorbed per unit area of fat surface (protein load).

It can generally be stated that although the same amount of energy has been transferred to the emulsion during emulsification, the properties of the emulsions differ considerably as a function of the emulsifying equipment, the power generated or the number of passes through the emulsification system. The emulsifying efficiency of the turbomixer is poorest in terms of creaming stability of the emulsions formed, whereas the ultrasonic device usually is the best choice of equipment at lower power input, and at higher power input the valve homogenizer is an equally good alternative, or even better.

When preparing the emulsions in a valve homogenizer the final fat surface area of the emulsions generally increases more as a function of power input, than as a function of number of passes. Distribution width, c_s , decreases mostly with increasing power supply and number of passes, but at the highest power input c_s starts to increase again. The protein load on the fat globules is largely determined by the fat surface area created and by the type of protein adsorbed. The soy proteins give a high protein load and the caseinates give a low protein adsorption at small fat surface areas created. This relation is, though, reversed at large surface areas of the fat globules. Salt addition to 0.2 M NaCl enhances protein adsorption at the fat globule interface in the case of soy protein and caseinate, but for the whey proteins protein load is higher in distilled water.

Both the interfacial and the emulsification studies suggest that the caseinates mainly cover the interface by adsorption of casein monomers from the bulk, whereas the soy proteins migrate to the interface by the gross associated complex, which disintegrates

at the interface in order to cover it. The whey proteins show an emulsifying and interfacial behavior somewhat intermediate to that of the other two proteins.

The results obtained in this study suggest that with variation of power input in a valve homogenizer, it is mainly the supply mechanism of protein molecules to the newly created fat interface, which determines the final particle size distribution of the emulsion. It is advantageous in order to get a large fat surface area to cover the fat interface slowly by adsorption from bulk. In repeated passes at constant supply of power, it is the resistance of the newly formed fat globule to withstand repeated homogenization, which is determining for the final particle size of the emulsion. In this case, the results indicate that it is the rate of building up the surface pressure, Π , at the fat globule interface, which governs the resistance to further disruption.

Lund, January 1978

Eva Tornberg

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REFERENCES

1. Hermansson, A.-M. Functional Properties of Proteins for Foods. Thesis. Lund, Sweden (1974).
2. Kinsella, J.E. Critical Reviews in Food Science and Nutrition, p. 219 (April) (1976).
3. Lankveld, J.M.G. and Lyklema, J. J. Coll. Interface Sci. 41, 475 (1972).
4. Dickerson, R.E. and Geis, I. "The Structure and Action of Proteins, Harper & Row, New York (1969).
5. Sophianopoulos, A.J. and van Holde, K.E. J. Biol. Chem. 239, 2516 (1964).
6. Lyster, R.L.J. J. Dairy Sci. 39, 279 (1972).
7. Koshiyama, I. Agr. Biol. Chem. 35, 385 (1971).
8. Badley, R.A., Atkinson, D., Hauser, H., Oldani, D., Green, J.P. and Stubbs, J.M. Biochem. Biophys. Acta 412, 214 (1975).
9. Koshiyama, I. and Fukushima, D. Cereal Chem. 50, 114 (1973).
10. Wolf, W.J. In "Soybeans - Chemistry and Technology" (Smith, A.K.; Circle, S.J., eds.) p. 93 (1972). Avi Publ. Co. Inc., Westport, Connecticut.
11. Koshiyama, I. J. Sci. Fd Agric. 23, 853 (1972).
12. Koshiyama, I. Agr. Biol. Chem. 32, 879 (1968).
13. Schmidt, D.G. and Payens, T.A.J. Surface and Colloid Sci. 9, 165 (1976).
14. Creamer, L.K. and Berry, G.D. J. Dairy Res. 42, 169 (1975).
15. Schmidt, D.G. and Buchheim, W. Neth. Milk Dairy J. 29, 17 (1975).
16. Boucher, E.A., Grinchuk, T.M. and Zettlemoyer, A.C. J. Coll. Interface Sci. 23, 600 (1967).
17. Graham, D.E. Thesis entitled "Structure of Adsorbed Protein Films and Stability of Protein Stabilized Foams and Emulsions". (1976). Unilever Research Laboratory, Colworth/Welwyn, The Frythe, Welwyn, Hertfordshire.
18. Böhm, J.Th.C. Thesis. Meded. Landbouwhogeschool, Wageningen, 74-5 (1974).
19. van den Tempel, M. Proc. 3rd Int. Congr. Surface Activity, Cologne, 2, 573 (1960).
20. Birdi, K.S. J. Coll. Interface Sci. 43, 545 (1973).

21. Evans, M.T.A., Mitchell, J.R., Musselwhite, P.R. and Irons, L. *Adv. Exp. Med. Biol.* 7, 122 (1970).
22. Adams, D.J., Evans, M.T.A., Mitchell, J.R., Phillips, M.C. and Rees, P.M. *J. Polymer Sci.* 34, 167 (1971).
23. Wu, L.C. and Bates, R.P. *J. Food Sci.* 37, 36 (1972).
24. Mitchell, J., Irons, L. and Palmer, G.J. *Biochem. Biophys. Acta* 200, 138 (1970).
25. Mulder, H. and Walstra, P. "The Milk Fat Globule. Emulsion Science as Applied to Milk Products and Comparable Foods", (1974). Pudoc, Wageningen and Commonwealth Agric. Bureaux, Farnham Royal.
26. Gopal, E.S.R. In "Emulsion Science" (Sherman, P., ed.). (1969). Academic Press, London.
27. Kiefer, P. *Z. Lebensmitteltechnologie und Verfahrenstechnik* 27, 12 (1976).
28. Treiber, A. *Z. Lebensmitteltechnologie und Verfahrenstechnik* 27, 11 (1976).
29. Phipps, L.W. *J. Dairy Res.* 41, 1 (1974).
30. Zieniuk, J. and Chivers, R.C. Measurement of Ultrasonic Exposure with Radiation Force and Thermal Methods. (1976). *Ultrasonics*, July.
31. Walstra, P. and Oortwijn, H. *Neth. Milk Dairy J.* 29, 263 (1975).
32. Walstra, P., Oortwijn, H. and de Graf, J.F. *Neth. Milk Dairy J.* 23, 12 (1969).
33. Matsumoto, S. and Fukushima, S. *J. Texture Studies* 5, 29 (1974).
34. Walstra, P. *Neth. Milk Dairy J.* 19, 93 (1965).
35. Walstra, P. *J. Colloid Interface Sci.* 27, 493 (1968).
36. van Ogden, L., Walstra, P. and Morris, H.A. *J. Dairy Sci.* 59, 1727 (1977).
37. Verwey, E.J.W. and Overbeck, J.T.G. "Theory of the Stability of Lyophobic Colloids". (1948). Elsevier Publ. Co., Amsterdam.
38. Lankveld, J.M.C. Thesis. Meded. Landbouwhogeschool, Wageningen. 70-21 (1970).
39. Walstra, P. *Neth. Milk Dairy J.* 23, 290 (1969).

FUNCTIONAL CHARACTERIZATION OF PROTEIN STABILIZED EMULSIONS: EFFECT OF PROCESSING

ABSTRACT

In order to evaluate the emulsifying characteristics of a protein the effect of processing of a protein stabilized soybean oil-in-water emulsion on the creaming stability of the emulsion was investigated. Three protein systems were used, namely soybean protein isolate, sodium caseinate and whey protein concentrate (WPC). The effects of four different types of emulsifying equipment, an ultra-turrax, a Sorvall homomixer, a valve homogenizer and an ultrasonic apparatus were studied. It was found that the type of emulsifying apparatus, the emulsifying time and intensity had a marked influence on the properties of the protein stabilized emulsions. It was demonstrated for all the types of apparatus and proteins used, that by increasing emulsifying intensity and time better emulsions, when characterized by the creaming method, are obtained up to a certain limit whereupon there is nothing to be gained by an additional increase of these factors. The necessary emulsifying intensity and time to obtain a certain stability range differed considerably according to the various proteins investigated as well as environmental conditions. This intensity-time dependence may be considered as an emulsifying characteristic of the protein.

INTRODUCTION

PROTEIN STABILIZED oil-in-water emulsions are to be found in various branches of the food industry. These include milk, cream, ice-cream, salad dressings, mayonnaise, gravies and meat emulsions. Ice-cream and meat emulsions differ from "true" emulsions as they contain one or two additional structures such as foam, suspension and gel. Moreover "true" emulsions can differ considerably in oil phase volume, in fat solid/liquid ratio and content and in viscosity of the continuous phase. This leads to different stability problems. It is, therefore, difficult to develop one single model system applicable in all cases for measuring the emulsification properties of a protein.

The determination of the emulsifying characteristics of proteins has evolved via two main approaches: emulsifying capacity and emulsion stability measurements.

The method of Swift et al. (1961) to investigate the emulsifying capacity is to determine the maximum amount of fat emulsified by a protein dispersion. Oil is added at a given rate to a protein dispersion being constantly stirred, until the emulsion inverts into a water-in-oil emulsion, as indicated by a sudden drop in emulsion viscosity. This method has been widely used, though modified in certain respects. The registration of the inversion point varies from visual appearance of the viscosity decrease (Swift et al., 1961; Pearson et al., 1965; Carpenter and Saffle, 1964) to change in amperage required to drive the blender (Crenwelge et al., 1974) and to change in electrical resistance of the emulsion (Webb et al., 1970; Satterlee and Free, 1973; Smith et al., 1973). Alternating blender speed from 1000 up to 20000 rpm has a large effect on both the amount of fat emulsified and the character of the emulsion produced. The amount of fat emulsified is also related to the

rate of oil addition and to the maximum temperature attained during emulsification (Swift et al., 1961). All these influencing factors raise doubts about the possibility of evaluating the field of application of a protein in food emulsions by this type of method.

There is a great difference between the emulsifying capacity and the emulsion stability produced with proteins. Emulsion stability is not a measure of maximum oil addition, but rather of the ability of the product to remain durable and unchanged.

Instability of an emulsion can visually appear as creaming and fat separation, which is usually caused by flocculation and coalescence, as shown in Figure 1.

Emulsion stability can be estimated from oil separation (Smith et al., 1973; Inklaar and Fortuin, 1969; Neelakantan, 1971). However, to be able to measure any fat separation from protein stabilized emulsions, "weak" emulsions have to be made — for instance by choosing an oil concentration near the inversion point — or stable emulsions have to be ultra-centrifuged. These criteria limit the research field, and it is questionable whether ultra-centrifugation provides a valid stability test for highly stable emulsions (Kitchener and Musselwhite, 1969). Time-consuming, but more informative, is to register the size distribution of the fat particles as a function of time (Mita et al., 1973; Kako and Sherman, 1974).

Another approach is to measure the extent of creaming as a rapid test to characterize the emulsion produced. Various parameters can be chosen to evaluate creaming, such as depth of cream layer, percentage of the total fat collected in the cream layer (Mol, 1963) or the percentage of the total fat left in the aqueous lower phase (Vakaleris and Sabharwal, 1972; Kurzhals, 1973). The percentage of water (Acton and Saffle, 1970, 1971) or of total solid (Smith and Dairiki, 1975) in the aqueous lower phase can also be recorded. Mild centrifugation is often used to accelerate the creaming rate of the fat particles. The tests vary depending on storage temperature and storage time of the emulsions.

Although the same stability test has been used by several authors it is difficult to compare the results, due to the fact that the formation of the emulsion has been made differently. It has been shown for milk that processing parameters strongly affect the character of the emulsion (Goulden and Phipps, 1969; Kurzhals, 1973; Mulder and Walstra, 1974; Walstra, 1975).

The aim of this work is to investigate the effect of processing of a protein stabilized soybean oil-in-water emulsion on the stability of the emulsion in order to obtain information on the evaluation of the emulsifying characteristics of a protein. Four different types of emulsifying equipment have been studied, as processing conditions may differ considerably for various types of food emulsions. They are an ultra-turrax, a Sorvall omni-mixer, a valve homogenizer and an ultrasonic apparatus. Three protein systems were used, a soy protein isolate (Promine-D), a sodium caseinate and a whey protein concentrate (WPC). In this paper low-viscous emulsions with a fat content of 40% are considered as model systems.

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MATERIALS & METHODS

Soy protein isolate

Promine-D (Central Soya), a commercially available sodium soybean proteinate. Analysis: protein ($N \times 6.25$) 89.6% (dry wt), solubility in distilled water at pH 9, denoted as (0-9), 65%.

Caseinate

Spray blend caseinate (DMV, Holland), a commercially available sodium caseinate. Analysis: protein ($N \times 6.37$) 89.3% (dry wt), fat 1.2% (dry wt), solubility in distilled water and in 0.2M sodium chloride solution at pH 7, denoted as (0-7) and (0.2-7), is 97.4% and 95.4%, respectively.

Whey protein concentrate (WPC)

WPC concentrated by gel filtration on an industrial scale was used. Analysis: protein ($N \times 6.25$) 65.6% (dry wt), fat 1.9% (dry wt). Solubility in distilled water at pH 9, denoted as (0-9), 45%.

Soybean oil

A commercially available soybean oil (AB Karlshamns Oljefabriker, Karlshamn, Sweden) was used. Analysis: fatty acid composition C 18:2 53.3%, C 18:1 23.0%, C 16 10.8%.

Preparation of samples

Protein dispersions composed of 2.5% (w/w) based on the protein content in distilled water or sodium chloride solution were made with the Sorvall omni-mixer. The pH was adjusted with 0.2M NaOH or 0.2M HCl. Soybean oil was added directly to the protein dispersion to attain 40% oil by weight.

Emulsion formation

Omni-mixer. A sample of 40g was emulsified in a stainless steel container with a capacity of 50 ml. A Sorvall omni-mixer, consisting of a rotating six-bladed knife suspended into the mixture, was used as a disperser. The rotor speed could be adjusted up to 15000 rpm.

Ultra-turrax. A quantity of 40g was emulsified in a 100 ml centrifuging tube of stainless steel with an Ultra-turrax type TP 18/2N (Janke & Kunkel) driven by a universal motor giving 20000 rpm. The motor speed was adjustable with a variable voltage transformer.

Ultrasonic Emulsions of 30g were formed in a 40 ml tube with a Branson Sonifier Cell Disruptor model B-12 (Branson Sonic Power Co.) consisting of a power supply, a sonic converter and a disruptor horn operating at a nominal frequency of 20 kHz. The power supply had a timing control, an activity output control with a dial marked in arbitrary units from 1 to 10. Before emulsification the mixture was shaken sufficiently to mix the two liquids, and thereafter the disruptor horn was suspended into the emulsion.

Valve homogenizer. Homogenization was accomplished on an aliquot of 50g with a single piston valve homogenizer equipped with a high pressure pump driven by a hydraulic system and having a pressure gauge and an adjustable valve opening (To be published). The mixture was shaken before it was poured into the feeder. Repeated homogenization was carried out.

Cooling was performed during all the emulsification procedures to keep the temperature of the emulsion near 25°C during processing.

Emulsion characterization. In this study the stability rating (SR) was determined on the basis of the percentage change of fat in the aqueous lower phase after creaming. The following equation was used:

$$SR = \frac{F_{\text{test}}}{F_{\text{original}}} \times 100 (\%)$$

F_{test} is the fat percentage of the bottom 5 ml of the sample, and F_{original} is the initial fat percentage of the whole sample. 30g of the emulsion were stored for 24 hr at 20°C. After storage the samples were centrifuged at room temperature at low speed ($180 \times G$) for 15 min. The centrifugation was supposed not to lead to any desorption or removal of proteinaceous material from the fat globule interface. Preliminary experiments with different storage times and temperatures and various centrifuging speeds were done to establish conditions giving reproducible results.

After centrifugation of the emulsion 5 ml of the lower phase was carefully removed with a syringe for fat determination by the Gerber method. The Gerber method was calibrated for the soybean oil over the range 0 - 50% (w/w) in the emulsions. No significant deviation of the fat content determination due to the various techniques of emulsion formation was evident.

RESULTS

Effect of different emulsifying equipment

The two mixers, the ultra-turrax and the omni-mixer, running at a maximum speed of 20000 rpm and 15000 rpm, respectively, were used to produce Promine-D (0-9) stabilized emulsions. The resulting stability rating as a function of the emulsifying time can be seen in Figure 2, which obviously shows that emulsions produced by the two mixers can differ considerably in creaming stability. This behavior was most pronounced at shorter emulsifying times, when the difference in creaming stability could be as high as 45%.

Comparisons between the ultra-turrax and the ultrasonic equipment can be made in Figures 3 and 5. The stability rating is plotted as a function of the intensity factor of the emulsifying apparatus; which for the ultra-turrax is the motor speed (emulsifying time of 2 min) and for the ultrasonic equipment is the power supply (emulsifying time of 1 min). The ultrasonic device seemed to be more efficient than the ultra-turrax.

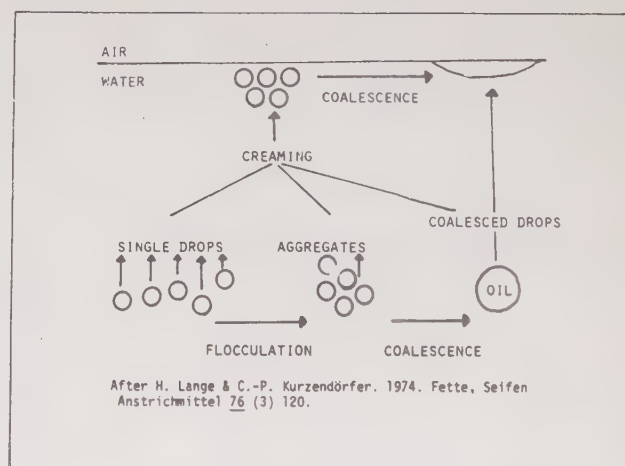


Fig. 1—A schematic representation of emulsion instability.

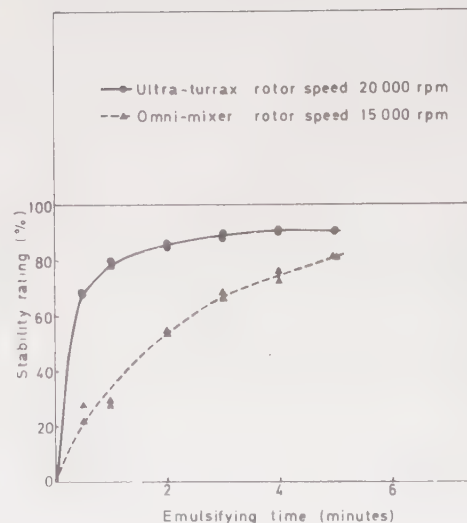


Fig. 2—Stability rating of a Promine-D (0-9) emulsion, emulsified with an ultra-turrax and an omni-mixer running at 20000 rpm and 15000 rpm, respectively, as a function of the emulsifying time.

The differences in creaming stability are pronounced for the WPC (0-9) and the caseinate (0.2-7) emulsions. It is also interesting to note that the WPC (0-9) emulsion had a higher stability score than the caseinate (0.2-7) emulsion when produced in the ultrasonic device, whereas the reverse was found when the emulsions were produced in the ultra-turrax.

The intensity factor of valve homogenization is the pressure drop and this has been varied for a caseinate (0.2-7) emulsion. The derived creaming stabilities when emulsifying for three minutes are plotted in Figure 6. This figure clearly shows that valve homogenization is more effective compared to the ultra-turrax in giving emulsions of high creaming stability.

Effect of emulsifying time and intensity

So far it has been shown that the type of emulsifying apparatus being used strongly influences the creaming stability of the protein emulsions. In determining the characteristics of the formed emulsion, the emulsifying time and intensity also play an important role, which is illustrated in Figures 2 to 7.

The dependence of stability rating on processing time can

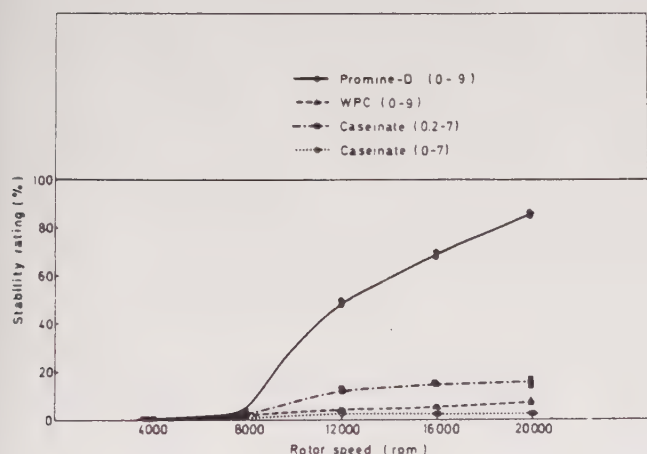


Fig. 3—Stability rating of different protein stabilized emulsions, emulsified for 2 min with an ultra-turrax, as a function of the rotor speed.

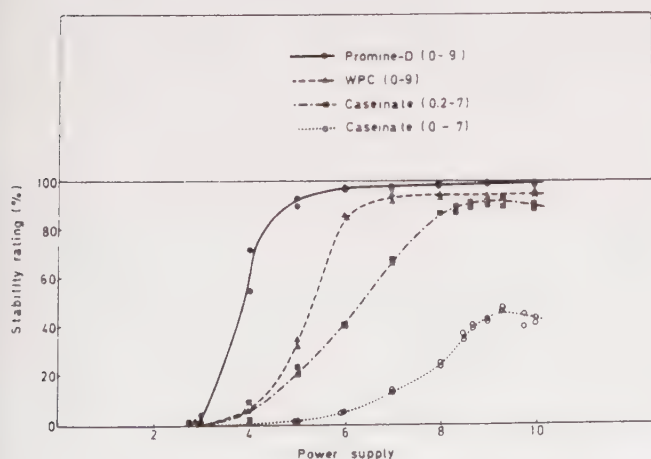


Fig. 5—Stability rating of different protein stabilized emulsions, emulsified for 1 min, as a function of the ultrasonic power supply.

best be seen in Figures 2 and 7. In Figure 7 caseinate dispersions of various ionic strength were used for emulsification in the valve homogenizer at two pressures, 7.5 and 15 MPa. In Figure 4 the resulting creaming stabilities of Promine-D (0-9) emulsions are plotted against various ultrasonic intensities, where the different curves represent various emulsifying times. The figures show that prolonged emulsification gave more creaming stable emulsions up to a certain limit whereupon nothing further could be gained in stability with time. The upper limit of stability rating is dependent on the apparatus used, the emulsifying intensity, the type of protein and environmental factors such as pH and ionic strength.

Increasing emulsifying intensity also gives rise to more creaming stable emulsions, which is clearly demonstrated in Figures 3, 4, 5 and 6 for all the types of apparatus and proteins used. In the case of emulsifying intensity a limit of creaming stability is also attained where the curve flattens out. This feature, however, varies according to the apparatus used, as can be seen in the case of caseinate (0.2-7): a stability rating of about 90% is achieved at the higher intensities with

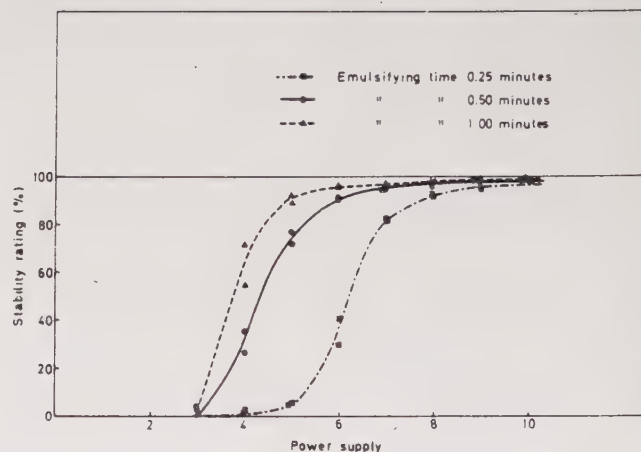


Fig. 4—Stability rating of a Promine-D (0-9) emulsion, emulsified for various times, as a function of the ultrasonic power supply.

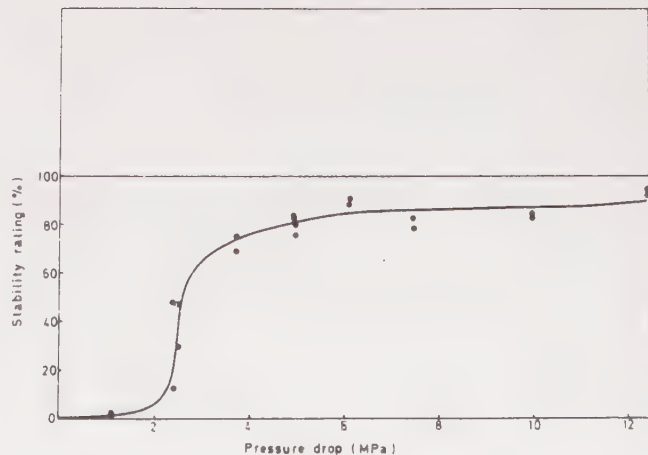


Fig. 6—Stability rating of a caseinate (0.2-7) emulsion, valve homogenized for 3 min, as a function of the pressure drop.

types of emulsifying apparatus with respect to their emulsifying efficiency, flow conditions, effect and energy input in the emulsifying unit must be controlled. These problems are under investigation.

REFERENCES

- Acton, J.C. and Saffle, R.L. 1970. Stability of oil-in-water emulsions. *J. Food Sci.* 35: 852.
- Acton, J.C. and Saffle, R.L. 1971. Stability of oil-in-water emulsions. *J. Food Sci.* 36: 1118.
- Carpenter, J.A. and Saffle, R.L. 1964. Simple method of estimating the emulsifying capacity of various sausage meats. *J. Food Sci.* 29: 774.
- Crenwelge, D.D., Dill, C.W., Tybor, P.T. and Landmann, W.A. 1974. A comparison of the emulsification capacities of some protein concentrates. *J. Food Sci.* 39: 175.
- Goulden, J.D.S. and Phipps, L.W. 1969. *Proc. 1st Int. Congr. Fd Sci. Technol.* 1962, 1, 765.
- Guy, E.J., Vettel, H.E. and Pallansch, M.J. 1972. Stabilization of milk fat/cheese whey emulsion. *Food Technol.* 26(2): 50.
- Inklaar, P.A. and Fortuin, J. 1969. Determining the emulsifying and emulsion stabilizing capacity of protein meat additives. *Food Technol.* 23: 103.
- Kako, M. and Sherman, P. 1974. The stability of imitation milks prepared from corn oil. *Milchwissenschaft* 29: 733.
- Kitchener, J.A. and Musselwhite, P.R. 1969. In "Emulsion Science," Ed. Sherman, P., p. 77. Academic Press, London.
- Kurzhals, H.A. 1973. Beurteilung des Homogenisier-effektes bei Milch. *Milchwissenschaft* 28: 637.
- Lange, H. and Kurzendörfer, C.-P. 1974. Zum Mechanismus der Stabilisierung von Emulsionen. *Fette, Seifen, Anstrichmittel* 76: 120.
- Lankveld, J.M.G. and Lyklema, J. 1972. Adsorption of polyvinyl alcohol on the parafin-water interface. *J. Coll. Interface Sci.* 41: 475.
- Mita, T., Yamada, K., Matsumoto, S. and Yonesawa, D. 1973. Dispersion state of protein-stabilized emulsions. *J. Texture Stud.* 4: 41.
- Mol J.J. 1963. *Mol. Off. Org.* FN255, 529.
- Mulder, H. and Walstra, P. 1974. The milk fat globule. Emulsion science as applied to milk products and comparable foods. Pudoc, Wageningen and Commonwealth Agric. Bureaux, Farnham Royal.
- Neelakantan, S. 1971. Studies on the emulsifying characteristics of some intracellular turkey muscle proteins. *J. Food Sci.* 36: 613.
- Pearson, A.M., Spooner, E.M., Hegarty, G.R. and Bratzler, L.J. 1965. The emulsifying capacity and stability of soy sodium proteinate, potassium caseinate and nonfat dry milk. *Food Technol.* 19: 1841.
- Satterlee, L.D. and Free, B. 1973. Utilization of high protein tissue powders as a binder/extender in meat emulsions. *J. Food Sci.* 38: 306.
- Smith, L.M. and Dairiki, T. 1975. Stability of milk fat emulsions. *J. Dairy Sci.* 58: 1249, 1254.
- Smith, G.C., Juhn, H., Carpenter, Z.L., Mattil, K.F. and Cater, C.M. 1973. Efficiency of protein additives as emulsion stabilizers in frankfurters. *J. Food Sci.* 38: 849.
- Swift, C.E., Lockett, C., and Fryar, A.J. 1961. Comminuted Meat Emulsions. The capacity of meats for emulsifying fat. *Food Technol.* 15: 468.
- Vakaleris, D.G. and Sabharwal, K. 1972. Stability of fluid food emulsions. *J. Dairy Sci.* 55: 277, 283.
- Walstra, P. 1975. Effect of homogenization on the fat globule size distribution in milk. *Neth. Milk Dairy J.* 29: 279.
- Webb, N.B., Ivey, F.J., Craig, H.B., Jones, V.A. and Monroe, R.J. 1970. The measurement of emulsifying capacity by electrical resistance. *J. Food Sci.* 35: 501.

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A Surface Tension Apparatus According to the Drop Volume Principle

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A new construction of a drop volume apparatus has proved to be successful in determining the interfacial tension for a variety of pure liquids as well as for solutions having surface tensions, which come to equilibrium quickly. Temperature dependence of the surface tension can easily be recorded even at elevated temperatures, which has been shown for water.

INTRODUCTION

The surface and interfacial tension is an important property associated with colloid systems. Comparisons of methods to determine the interfacial tension are given by Padday (1, 3), Drost-Hansen (2), and Boucher *et al.* (4).

Very rarely used for interfacial tension measurements is the capillary rise method, because of problems concerning the contact angle and the adsorption to the glass wall (2). Moreover, it is very difficult to get capillary tubes of uniform bore (2). The ring method can be used for measuring the surface tension of pure liquids, but this method is not convenient for interfacial tension determinations, because of variations of the contact angle between the interface of the two liquids and the solid ring (1). The same problem arises, when measuring the interfacial tension with the Wilhelmy plate method. The drop volume and the pendent drop methods do not suffer from this disadvantage. They can both be considered as measurements of a hanging drop, as suggested by Boucher and Evans (5). The pendent drop method studies the drop at various stages of growth, whereas the drop volume method determines the amount detached. The accuracy of the pendent drop

method is, however, limited, because the dimensions of the drop at various stages of growth are difficult to determine precisely (4). Providing the following conditions are met, the drop volume is considered to be very accurate and reproducible for measuring interfacial tensions between two liquids:

(a) The orifice is sharply cut and of an accurately known radius.

(b) The orifice is carefully ground and fully wetted.

(c) The drop is made to detach itself as slowly as possible.

The aim of this work is to show a new design of a drop volume apparatus, for accurate determinations of surface and interfacial tensions and its temperature dependence.

EXPERIMENTAL

Materials

The water was first treated with activated carbon overnight and then doubly distilled from alkaline permanganate in all-Pyrex glass. The conductivity of the water was approximately 1.6×10^{-6} mho/cm. For the determination of surface tension of water at temperatures above room temperature, solved air

was removed to avoid air bubbles during the measurements. All glassware was washed first with sulfuric acid-dichromate solution and then with double-distilled water.

Benzene was of spectroscopic grade, and redistilled hexane for gas chromatographic work was used.

Benzene, hexane, and diethyl ether were all treated with activated carbon for 12 hr. The carbon was filtered off in a glass filter. In case of interfacial tension measurements between two pure liquids, they were left in contact overnight to reach equilibrium.

The solutions of KCl were prepared by dissolving KCl (p.a.) in double-distilled water.

Methods

Density measurements were carried out using a pycnometer with a volume of 25 ml at 20°C.

The surface tension apparatus comprises: the electronic driving and recording device; the recorder; the glassware unit; and the thermostating box.

The crucial part is the glassware unit shown in Fig. 1 consisting of a screw (8), the rotation of which causes the penetration of a piston (6), and the formation of a drop from a brass tip (4). The screw is rotated by means of a step motor (10); the motion of the step motor is transmitted through a gear box (9). The liquid of the highest density is held in the syringe, whereas the lower density liquid is in the glass box (3). When measuring at elevated temperatures the opening in the glass box is connected to another thermostatted glass box filled with the same liquid as in the syringe in order to have a saturated atmosphere in the case of an air/liquid interface. The temperature of the liquid in the syringe and in the glass box (3) is maintained ($\pm 0.10^\circ\text{C}$) by circulating water through the jacket (1). The tip is of brass and ground to achieve good wetting ability (6). As the form of the tip should be as nearly circular as possible, it was ground on a precision lathe.

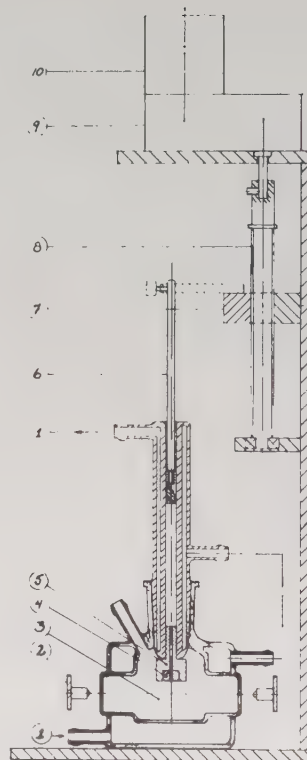


FIG. 1. Construction drawing of the glassware unit.

Roughening of the lower face of the tip was carried out periodically to secure good wetting. To prevent "creep" of the liquid up the outside of the tip, it was covered by a Teflon ring (5). This construction also facilitates the changing of tips when working with different liquids and interfaces in order always to get the optimum form of the drop according to the correction term (see below). One can easily exchange the tip of the syringe, because Teflon is malleable at room temperature. The level of the glassware unit is adjustable with screws, so that the end of the tip can be set horizontally. A photocell (2) is incorporated to sense the detachment of the drop, and a signal is given to the recorder.

The step motor (10) is connected to an oscillator placed in the electronic device. The pulses generated by the oscillator are recorded on a counter display, allowing digital reading of the volume extruded. The rate of

TABLE I

Interfacial Tension at 20°C Measured with the Drop Volume Apparatus Compared to Data from Literature

Interface	Interfacial tension (drop volume) (mN m ⁻¹)	Interfacial tension (literature) (mN m ⁻¹)
Water/air	72.74 ± 0.18	72.75 (6)
Water/hexane	50.71 ± 0.17	51.10 (8), 50.1 (9)
Water/benzene	33.35 ± 0.06	34.96 (6), 33.9 (9)
Water/diethylether	10.81 ± 0.10	10.70 (8)

drop formation is controlled by adjustable pulse rates (from 1 to 240 Hz) and exchangeable screws with different pitches.

The determination of the volume extruded per pulse was carried out by weighing drops of mercury formed in air at 20 and 50°C. Taking into account the small variation in volume per pulse with temperature, the resultant calibration constants of the two different screws were:

Screw with a fine pitch: $(5.38 \pm 0.02) \times 10^{-6}$ cm³/pulse,

Screw with a coarse pitch: $(2.15 \pm 0.01) \times 10^{-5}$ cm³/pulse.

Outline of Procedure

The relation $V_{\text{tot}}\Delta\rho g = 2\pi r\gamma$ states that the volume of a drop is proportional to the radius r of the tip and to the surface tension (γ). Harkins and Brown (6) claimed that the form or the shape of the maximum hanging drop determines the quantity of the falling drop. They deduced by an extensive series of experiments the ratio of the volume of that part of the drop that falls (V) to the total volume (V_{tot}) below the orifice calculated according to the relation above. The fraction falling was found to depend solely on the value $r/V^{1/3}$ or r/a , where a is the capillary constant. For the calculation of the true surface tension from the volume (V) of a falling drop, a modified equation should be used:

$$\gamma = V\Delta\rho g / 2\pi r f(r/V^{1/3}).$$

$\Delta\rho$ is the difference in densities of the two phases and g is the acceleration due to gravity.

Dropping tips should preferably be chosen so that $f(r/V^{1/3})$ is around 0.600 for liquid/air interfaces and about 0.625 for liquid/liquid interfaces, according to Harkins and Brown (6). They considered $f(r/a)$ to be more accurate than the alternative $f(r/V^{1/3})$ correction factor. Since $f(r/V^{1/3})$ is easier to obtain and always equal to $f(r/a)$ for any drop, it will only be necessary to know the relation between r/a and $r/V^{1/3}$ in order to change the correction factor. A fourth-power polynomial relationship derived by Wilkinson (7) has been used in this connection.

The drops require a slow growth during the last stages of its formation, as a drop formed rapidly is heavier than one formed slowly, because the drop is "blown up" with more liquid coming down the tube. Therefore the first 90% of the drop volume is formed at a rate of 240 Hz with the screw of fine pitch, whereas the rate of formation is exchanged to 10 Hz for the last 10% of the drop.

RESULTS AND DISCUSSION

Surface and Interfacial Tensions of Pure Liquids

To demonstrate the performance of the apparatus for interfacial tension measurements, the interfacial tension for a wide variety of liquids, covering interfacial free energies from 70 down to 10 mN m⁻¹ were recorded at 20°C. The results are shown in Table I. The accordance with the literature data is satisfying, and the errors of the measurements are of the same magnitude as for the calibration constant.

TABLE II

Surface Tension of KCl Solutions at 20°C Measured with the Drop Volume Apparatus Compared to Data from Literature

Interface	Surface tension (drop volume) (mN m ⁻¹)	Surface tension (literature) (mN m ⁻¹)
0.15 M KCl/air	73.11 ± 0.34	73.1 (8)
1 M KCl/air	74.34 ± 0.24	74.2 (8)

TABLE III

Surface Tension of Water at Different Temperatures Measured with the Drop Volume Apparatus Compared to Data from Literature

Temperature (°C)	Surface tension (drop volume) (mN m ⁻¹)	Surface tension (literature) (mN m ⁻¹)
20	72.74 ± 0.18	72.75 (6)
25	71.91 ± 0.16	71.97 (8, 11), 71.95 (10)
30	70.91 ± 0.21	71.18 (8), 71.10 (10), 71.16 (11)
40	69.39 ± 0.20	69.56 (8), 69.27 (10), 69.42 (11)
50	67.70 ± 0.33	67.91 (8), 67.54 (11)

Surface Tension of KCl Solutions

To show the applicability of the apparatus for a two-component solution, which reaches the equilibrium structure in the surface layer very quickly, solutions of KCl were chosen. The surface tension of water increases when adding salt to it. In Table II the measured surface tensions of 0.15 and 1.0 M KCl solutions at 20°C are collected, and the agreement with available literature data is good.

Temperature Dependence of Surface Tension of Water

The surface tension as a function of temperature can for some methods be difficult to measure, especially at higher temperatures. Johansson and Eriksson (10) have measured the surface tension of water within the temperature range 5–40°C by means of the Wilhelmy plate method. They noted for $t > 25^\circ\text{C}$ errors in the determination of the surface tension, due to increased water evaporation. The water level position relative to the mica blade was changed at the higher temperatures, and the perimeter of the mica blade

at the meniscus may not be exactly the same at different heights, which is why an error can be induced in the surface tension determinations (10). In order to eliminate these difficulties they built a special sample container.

The drop volume method does not suffer from this disadvantage, and data for the surface tension of water between 20 and 50°C have been measured and compared to data available in the literature. These data can be seen in Table III, and the results obtained here coincide well with literature values.

ACKNOWLEDGMENTS

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REFERENCES

1. PADDAY, J. F., in "Surface and Colloid Science" (E. Matijevic, Ed.), Vol. 1, p. 39. Interscience, New York, 1969.
2. DROST-HANSEN, W., *Ind. Eng. Chem.* **57**, 38 (1965).
3. PADDAY, J. F., and RUSSEL, D. R., *J. Colloid Sci.* **15**, 503 (1960).
4. BOUCHER, E. A., GRINCHUK, T. M., and ZETTLEMOYER, A. C., *J. Colloid Interface Sci.* **23**, 600 (1967).
5. BOUCHER, E. A., and EVANS, H. J. B., *Proc. Roy. Soc. A* **346**, 349 (1975).
6. HARKINS, W., and BROWN, F. E., *J. Amer. Chem. Soc.* **41**, 499 (1919).
7. WILKINSON, M. C., *J. Colloid Interface Sci.* **40**, 14 (1972).
8. WEAST, R. C., and SELBY, S. M. (Eds.), "Handbook of Chemistry and Physics," pp. F-25, F-27. Chemical Rubber Co., Cleveland, Ohio, 1966.
9. GLASS, J. E., *J. Polym. Sci.* **34**, 141 (1971).
10. JOHANSSON, K., and ERIKSSON, J. C., *J. Colloid Interface Sci.* **40**, 398 (1972).
11. HARKINS, W. D., and ALEXANDER, A. E., in "Physical Methods of Organic Chemistry" (A. Weissberger, Ed.), 3rd ed., Vol. 1, Part 1. Interscience, New York, 1959.

THE APPLICATION OF THE DROP VOLUME TECHNIQUE TO MEASUREMENTS OF
THE ADSORPTION OF PROTEINS AT INTERFACES

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Abstract

A new procedure for the application of the drop volume technique to measurements of the rate of adsorption of proteins at interfaces has been developed. The mode of adsorption of the proteins lysozyme, β -lactoglobulin and bovine serum albumin (BSA) at the air-water interface has been measured with the drop volume method and has been compared to measurements with the Wilhelmy plate technique. Due to surface enlargement of the drop throughout the process of the surface tension decay, slower kinetics of the adsorption process is obtained by the drop volume method compared to the Wilhelmy plate technique, and the proteins investigated were differently sensitive to this surface expansion. The adsorption process of the proteins has been evaluated in terms of different rate-determining processes.

Introduction

The interfacial tension (γ) is an important factor in the formation of emulsions and foams, although it has been pointed out by van den Tempel (1) that it is not so much the absolute value of γ which is crucial, but rather the gradients in γ that arise during the emulsification or foaming process.

The choice of the appropriate technique to measure the interfacial tension depends on the relationship between the phenomenon to be investigated and the information that the method can give. In the specific case of proteins stabilizing foams and emulsions, the kinetics of adsorption are of particular interest, and thus techniques which give dynamic rather than equilibrium measurements of γ , give results which are more relevant to emulsification and foaming.

There are few comparisons of available techniques for interfacial tension determinations reported in the literature, especially for the case of time-dependent interfacial tension (2 - 5). The ring method is unsatisfactory for time-dependent

solutions, as it can give errors as large as 10 mN m^{-1} (2), and one main source of error is probably the act of detachment of the ring during measurement (3). The heights obtained in the capillary-rise method are found to be affected by aging of the surfaces over considerable periods of time (3). Both Boucher et al. (4) and Addison and Hutchinson (5) have compared the Wilhelmy plate, the drop volume and the pendent drop methods to measure the surface tension decay of surfactant solutions. Boucher et al. (4) found the Wilhelmy plate, the drop volume and the pendent drop methods to give almost the same values of the equilibrium surface tensions of surfactant solutions. On the other hand Addison and Hutchinson (5) found a difference between the rate of adsorption and the equilibrium values obtained by the pendent drop and the Wilhelmy plate technique. Lower γ values were found by the latter technique. The γ -t-curves obtained with the drop volume and the pendent drop methods coincide, which is reasonable since these methods are virtually the same, as discussed elsewhere (6, 7).

The aim of this article is to demonstrate the applicability of the drop volume technique to the elucidation of the mode of adsorption of proteins, and to compare it to the Wilhelmy plate technique. The following procedure was used. A drop of a certain volume, corresponding to a certain γ -value, is expelled rapidly, and the time necessary for the surface tension to fall to such a value that the drop becomes detached is measured. Due to the surface tension decay from the time of formation until detachment of the drop, the surface of the drop expands. The adsorption behavior of a protein can, in this case, be considered to be similar to adsorption at a newly created surface in a foam or an emulsion, with no further disruption of the interface.

In this paper, only surface tension measurements at the air-water interface are given, and they are compared to surface tension data obtained by the Wilhelmy plate method. The proteins lysozyme, β -lactoglobulin and bovine serum albumin (BSA) were chosen for this study because their surface behavior from solution has already been subject to considerable study (8 - 17).

Experimental

Materials

The method used for water purification is described in detail elsewhere (6).

Three times recrystallised egg-white lysozyme, once recrystallised BSA and β -lactoglobulin (A and B) from Sigma Chemical Co. were all lyophilized, and used without further purification. The three proteins were dissolved in a phosphate buffer, pH of 7 and an ionic strength of 0.1, as described elsewhere (16). The protein concentration used were $10^{-2}\%$ (w/w).

In the investigation of the concentration dependence of the surface tension of lysozyme solutions, the solutions were prepared by dissolving lysozyme in 1 M KCl. For concentrations less than 0.1% (w/w), the solutions were prepared by diluting the stock solution of 0.1%. Acidity was adjusted to pH 3 with HCl.

All protein solutions were freshly made each day.

Methods

Density measurements of the solutions were carried out as described earlier (6). The description of the surface tension apparatus and its mode of operation according to the drop volume principle are given elsewhere (6).

Outline of procedure

The procedure for making measurements with pure liquids and solutions that come to equilibrium quickly has been described earlier (6). For measurements of time-dependent surface tensions a modified procedure is necessary. Part of the total drop does not fall, and the remaining portion will have its surface tension altered by adsorption of a surface active material; Hence, this aged surface must be eliminated when the next drop is to be formed. Thus, by forming one or two drops

rapidly, the next drop to be measured should be free of aged surface. The rapidly formed drops should have a rate of drop formation such that the liquid flow down the tube does not influence the resulting volume of the drops. This technique is limited to slow adsorption processes, as in the case of protein adsorption, because the rate of adsorption of the surface active material at the interface should be negligible compared to the time of formation of the drops, which is approximately 20 s at the air-water interface.

Because different drop sizes give rise to residual volumes of differing quantities, the residual volume (v_r) below the orifice of the rapidly formed drop must be calculated in order to know the volume of the following drop. This entails the calculation of the maximum volume (v_m) before the falling part (v_d) breaks away, and the measurement of the detached volume (v_d). Harkins and Brown (18) calculated v_m of an "ideal" drop according to the relation $v_m \Delta \rho g = 2\pi r \gamma$. This relation is, however, not valid, because (i) it does not take into account the fact that surface tension forces do not always act at an angle of $\phi = 90^\circ$ to the tip, and (ii) it also neglects the downward force due to the excess pressure inside the drop (7, 19). To avoid these errors the equation describing the volume of a pendent drop can instead be used to compute the maximum volume below the orifice just before it becomes detached. This has recently been done in reduced terms, $V_m = v_m/a^3$, by Boucher and Evans (7) as a function of $X = r/a$, where $a^2 = 2\gamma/\Delta \rho g$.

Harkins and Brown (18) claimed that the volume of the falling fraction (v_d) of the maximum hanging drop (v_m) is determined by the shape of the drop, and this in turn depends solely on the value $r/v_d^{1/3}$ or r/a . It may be argued that the break away process should be analysed in terms of dynamics of flow during detachment before this assumption can be justified. Unfortunately, this analysis involves extremely complicated computational problems (21), and therefore Pearson and Whitaker (20) and Whitaker (21) have used another approach to avoid these problems. By means of photographic evidence Pearson and Whitaker (20) showed

that the break away process for a 1.5×10^{-2} molar heptanoic acid solution was essentially identical to that exhibited by water. Whitaker (21) has studied the stability of a cylindrical column of liquid as a model for the break away process, and the results obtained suggest that the drop stability is independent of the rheological properties of the surface, and that the geometry of the break away process depends only on v/r^3 ; this is in accordance with the experimental findings of Harkins and Brown (18).

On the basis of these recent findings the following procedure has been worked out. The detached volume v_d of a quickly formed drop is measured, and from this volume the dimensionless quantity $r/v_d^{1/3}$ is calculated. For a given liquid there is only one unique value of $r/a = X$ for each value of $r/v_d^{1/3}$, and Wilkinson (22) has derived a fourth-power polynomial relationship between these quantities, which is used in this study. Using the tables given by Boucher and Evans (7) the value of X corresponding to $r/v_d^{1/3}$ gives the maximum pendent drop volume in reduced terms, $V_m = v_m/a^3$. The residual volume (v_r) can then be calculated according to $v_r = v_m - v_d$. The next drop to be measured is extruded by adding a certain amount of liquid, v_s . The total amount of this new drop hanging beneath the tip is then obtained from the sum of v_s and v_r . A new dimensionless quantity r^3/v_m is formed, and this can give the corresponding value of X by use of the tables of Boucher and Evans (7). The surface tension can then be determined from the equation for the capillary constant, a :

$$\gamma = \frac{\Delta \rho g r^2}{2X^2} \quad [1]$$

The comparison between the two methods of determining the interfacial tension was performed at 25°C, whereas the measurements on the lysozyme solutions at different protein concentrations were made at 20°C.

Theory applied to protein adsorption

It is reasonable to assume that any process responsible for the time dependence of the reduction in surface tension by a polymer molecule must involve an increase in the number of adsorbed segments per unit area with time (23 - 25).

In the case of protein molecules adsorbing at interfaces, Graham (14, 26) has shown that the primary layer of molecules is largely responsible for determining Π , and the influence on Π of an increase in the film thickness during adsorption can therefore be assumed to be negligible.

The rate of reduction of interfacial tension is determined by three consecutive or concurrent processes (14, 27): (1) the diffusion of whole protein molecules to and attachment at the interface; (2) spreading or unfolding of already adsorbed molecules; (3) molecular rearrangements and reconfigurations of adsorbed molecules. The two latter mechanisms involve transport of molecular elements or protein segments on the surface.

A useful analysis of the kinetics of adsorption derives from the work of Ward and Tordai (28, 29), who considered the effects of diffusion from the bulk liquid to the surface and the energy barrier which the molecule must overcome in order to be adsorbed. They were able to show that this mechanism could describe the kinetics of adsorption of fatty acids at the hexane/water interface.

In the absence of convection and desorption, the number of molecules per unit area (n) adsorbed at time t , providing the process is diffusion-controlled, is given according to Ward and Tordai (28) by:

$$n = 2 C_{pr} \left(\frac{Dt}{3.142} \right)^{1/2} \quad [2]$$

The quantity D is termed the diffusion coefficient. It will only be equivalent to the conventional diffusion coefficient if there is no energy barrier between

the surface and the subphase, and this is the case only at the very beginning of the process. In the general case, the value of D will be a quantity characteristic of both diffusion and the crossing of the barrier.

In the case of protein adsorption the reduction of interfacial tension will be related to the number of active groups of protein segments adsorbed. If ν is the number of active groups per molecule, νC_{pr} should be used for the concentration of active groups (ag). By analogy, the number of molecules per unit area, n , will instead be n_{ag} , i.e. the number of adsorbed active groups per unit area.

When the modified form of equation [2] is combined with the simple two-dimensional equation of state $\frac{\Pi}{n_{ag}} = \frac{kT}{\nu}$, which is considered to be obeyed in the relatively low pressure region, where diffusion is the rate-determining step (30), we obtain:

$$\gamma = \gamma_0 - 2 C_{pr} kT \left(\frac{D}{3.142} t \right)^{1/2} \quad [3]$$

A plot of γ against $t^{1/2}$ will then be linear.

When a sufficient high energy barrier exists, the diffusion will no longer be rate-determining, but the rate of segment, or protein, penetration into the surface film will be rate-limiting.

Ward and Tordai (29) considered this barrier to consist of the work $\Pi \Delta A$, done in creating a space of area ΔA in a surface film of surface pressure Π in order to adsorb a molecule. They formulated a differential equation for the kinetics of adsorption, and if we assume irreversible adsorption the equation is:

$$\frac{dn}{dt} = k_1 C_{pr} \exp [- \Pi \Delta A / kT] \quad [4]$$

In this equation n is the concentration of the solute adsorbed (number of molecules per unit area), and k_1 is the rate constant of adsorption.

MacRitchie and Alexander (8) have used this equation for the adsorption of protein molecules from the bulk to the interface. This work has been commented on by Bull (31) who considered it more likely that the decay of the surface tension with time is not entirely due to the adsorption of new protein molecules to the surface, but also involves the expansion and rearrangement of the protein molecules on the surface.

To take into account the various processes that can occur during the surface tension decay, equation [4] can be modified. By assuming that γ reflects the level of adsorption of protein segments and/or active groups (ag), dn_{ag}/dt can be considered to be proportional to $\frac{d\Pi}{dt}$. The effective concentration of ag is now given by νC_{pr} . Equation [4] will consequently be modified to give eqn. [5]:

$$\ln \frac{d\Pi}{dt} = \ln (\bar{k}_1 \nu C_{pr}) - \Pi \Delta A_{ag} / kT \quad [5]$$

If ΔA_{ag} is assumed to be constant, then a plot of $\ln \frac{d\Pi}{dt}$ versus Π should be linear with a slope giving ΔA_{ag} , which will represent the mean area created in the film in order to adsorb an active group in the protein molecule. The values of ΔA_{ag} , ν , $\bar{k}_1$ and C_{pr} can be considered to be constants within each rate-determining process, but these values will change when the rate-determining process is changed. When the rate is determined by diffusion (eqn. [3]) $\ln \frac{d\Pi}{dt}$ is not formally a linear function of Π as in eqn. [5]. It was found, however, for all practical purposes and for the range of Π -values of interest here, that $\ln \frac{d\Pi}{dt}$

can be represented by eqn. [5]. When $\ln \frac{d\Pi}{dt}$ is independent of Π , i.e. when $\Delta A_{ag} \approx 0$, this is taken as an indication of only rearrangement processes taking place. No new areas, ΔA_{ag} , are created in the film, but the surface coverage is still enhanced by protein segments adsorbing at the surface unoccupied by protein segments in the preceding processes. Eqn. [5] is expected to be valid as long as the effect of collapse or coagulation is negligible.

Results and discussion

In Figure 1 the interfacial tension has been plotted as a function of time up to 40 minutes for the three proteins investigated, using both the drop volume and the Wilhelmy plate method. The initial interfacial tension (γ_0) equal to the interfacial tension of the buffer solution, was measured to be $72.4 \pm 0.4 \text{ mN m}^{-1}$. In the case of the three proteins studied here, the drop volume method indicated slower kinetics than obtained with the Wilhelmy plate, which is in accordance with an enlargement of the surface of the drop throughout the process of the surface tension decay. Because of the large flat surface in the Wilhelmy plate method, eddy and convective currents more frequently occur than in the drop volume method, which can also contribute to the differences in the γ -t-curves obtained. It is interesting to compare the surface behavior of these proteins when the surface is enlarged. As can be seen from Figure 1, the γ -t-curve of β -lactoglobulin is not as sensitive to the surface expansion, whereas the rate of the surface tension depression by BSA and lysozyme is greatly diminished by the expanding surface in the drop volume method.

In Figure 2 γ , read off from the fitted curve in Figure 1, has been plotted against $t^{1/2}$ for lysozyme, β -lactoglobulin and BSA according to equation [3]. There is an initial period before a linear relationship is established for the measurements with the drop volume method, and this is especially pronounced for lysozyme. This phenomenon can probably be attributed to the enlargement

of the surface of the drop, accomplished by the surface tension decay of the first arrived molecules, being faster, than the process of building up an activation barrier against further adsorption, which is also a consequence of increasing Π . Therefore the rate of arrival of protein segments or molecules at the surface, as reflected in $\frac{d\Pi}{dt}$, increases during this initial period as a function of Π . This is shown by the results for lysozyme given in Figure 3, where $\log \frac{d\Pi}{dt}$ is plotted as a function of Π . The maximum slope, which corresponds to the linear part of the plots marked with two arrows given in Figure 2 is always taken for quantitative interpretations. It is assumed that the supply of protein molecules by diffusion is the limiting factor during this stage. After the linear part of the $\gamma-t^{1/2}$ curve, the rate of decrease in surface tension with increasing $t^{1/2}$ falls off.

In Figure 3, $\log \frac{d\Pi}{dt}$ has been plotted against Π for the three proteins, measured with the Wilhelmy plate and the drop volume methods. The slopes were obtained by computer differentiation of the fitted curves of Figure 1, which were approximated by parabolas for small sections. Linear parts derived by linear regression analysis were obtained with evident breaking points for all the protein solutions. The correlation coefficients for the linear parts obtained could vary from 0.70 to 0.99. The linear behavior in the plots indicates that the theory on which eq. [5] is based, is not invalid, and that the rate of desorption is negligible. The graphs of Figure 3 show that the surface behavior of proteins has a step character, more or less obvious, and that in some cases the abrupt change from one step to the consecutive one is highly indicative of a cooperative process taking place at the interface.

To summarize the quantitative evaluation of the graphs from Figures 2 and 3, the surface pressure (Π_{att}) and ΔA_{ag} attained during the different rate-determining steps are collected in Table I. ΔA_{ag} was evaluated from the

slopes of all the linear parts in Figure 3 according to eq. [5]. $\frac{\Delta t_{att}}{40}$ and $\frac{\Delta \Pi_{att}}{\Pi_{40}}$ are quotients, which indicate, respectively, the relative contribution of the time elapsed and Π_{att} of each rate-determining step in relation to 40 minutes and to the surface pressure attained after 40 minutes.

Surface pressures attained during the "diffusion" steps in Table I, denoted as $\Pi_t^{1/2}$, evaluated from the curves in Figure 2, and the surface pressures for the first steps derived from Figure 3, denoted as Π_A , are the same within the limits of error, which implies that it is possible to distinguish when diffusion or penetration of the surface film is the rate-controlling factor. Unfortunately, the diffusion step is too fast to be detected with the Wilhelmy plate method (see Figure 2), so these values are only estimates. The surface pressures attained during the initial step, when the effect of surface enlargement is evident, also coincide well, when evaluated from Figures 2 and 3 for the interfacial adsorption of lysozyme.

Diffusion controlled adsorption of proteins at an interface can imply either that the molecules diffuse to the interface and adsorb without further spreading, or that spreading or unfolding is so rapid that diffusion becomes the rate-controlling factor. The penetration step may involve either spreading or unfolding of already adsorbed molecules or adsorption of additional molecules arriving at the interface. In order to be adsorbed, however, a surface area, ΔA_{ag} , has to be cleared in the film, which requires molecular rearrangements of the already adsorbed protein molecules. Graham (14) has been able to show the existence of two kinetic regions during the penetration-controlled adsorption of β -casein, BSA and lysozyme, the first one describing the adsorption when additional molecules can arrive at the interface, the second one involves only rearrangements within the film with no further adsorption of molecules from the bulk.

Although the results presented in table I have only been recorded up to 40 minutes, two discrete penetration steps with discernible activation barriers can be observed for the proteins studied, anyhow, when measuring with the Wilhelmy plate method. Comparing the two penetration steps, the first one is always faster and requires a smaller area, ΔA_{ag} , to be swept out in the film, whereas the second one is slow and the rearrangements occur with a greater number of residues, i.e. with a larger ΔA_{ag} . This is consistent with the larger the surface area, ΔA_{ag} , to be cleared, the more number of residues to be rearranged within the surface film, and the slower is the process. Graham (14) has shown that rearrangements occur with a greater number of segments in a protein film with greater structure and order. This is in accordance with ΔA_{ag} increasing with Π . The last step occurring for β -lactoglobulin, shown in Figure 3, when using the drop volume method, gives $\log \frac{d\Pi}{dt}$ independent of Π , which is taken to indicate the occurrence of rearrangement processes taking place, with no need of new areas to be swept out in the interface. Further support for this assumption is that this rearrangement step follows directly after a penetration step with a high value of ΔA_{ag} .

The results in Table I indicate that the supply of protein molecules or segments to the interface is mainly diffusion controlled in all cases (compare the values of $\frac{\Delta \Pi_{att}}{\Pi_{40}}$), but the rate of diffusion differs considerably due to the protein adsorbed. Comparing the values of the quotient $\frac{\Delta t_{att}}{40}$, the diffusion of β -lactoglobulin is most rapid followed by BSA and lysozyme, the latter having a comparatively slow diffusion. Evidently, a slow diffusion controlled adsorption, as in the case of lysozyme, seems to be a condition for the effect of surface enlargement to become evident. The slow diffusion of lysozyme in comparison with the two other proteins seems to be contradictory, as the molecular weight of lysozyme ($\approx 14\ 600$) is smaller or in the same order of magnitude as for β -lactoglobulin ($\approx 18\ 500$), BSA having the highest molecular weight ($\approx 66\ 000$). Considering not only the diffusion coefficient of the molecules, but also the probability of successful collision with the interface resulting in adsorption,

as variables governing the diffusion-controlled adsorption, electrostatic hindrance during adsorption and the hydrophobicity of the molecules (14) have to be taken into account. As lysozyme is far away from the iso-electric point, the net charge of the molecule is high; hence the electrostatic hindrance during adsorption will probably contribute to a diminished number of successful collisions, being one of the reasons why diffusion of lysozyme is most slowly of all the proteins studied. Evidently the probability of successful collision of molecules with the interface is of great importance in making the diffusion-controlled process fast, which also has been reported by Graham (14).

Comparing the two methods of measurements, the first to be noted is that the effect of surface enlargement during the initial step of diffusion does not occur with the Wilhelmy plate method. The next is that the diffusion-controlled step mostly last to a higher surface pressure, Π , and is much faster, when using the Wilhelmy plate than the drop volume method. This is especially obvious for BSA. Probably due to the surface renewal during the surface tension decay in the drop volume method, the diffusion step is slowed down, and the unfolding step is promoted. Both these phenomena enhance the possibility of concurrent unfolding or spreading of adsorbed molecules during the diffusion-controlled step, when measuring with the drop volume method, which will lead to a build up of an adsorption barrier at an earlier stage of the adsorption process, i.e. at a lower Π . This reasoning will hold as long as unfolding or spreading of molecules gives rise to lowered compressibility of the surface film. For lysozyme this behavior is not observed, which can be attributed to its low capability to unfold at an interface, which has been demonstrated by Graham (14). It is also interesting to note that the diffusion of β -lactoglobulin is comparatively rapid and is not much slowed down, when measuring with the drop volume method. This could be one of the reasons, why the γ -t-curve obtained with the drop volume method is not so much altered compared to the curve obtained with the Wilhelmy plate method, as seen in Figure 1.

To illustrate the concentration dependence of 1 M KCl solutions of lysozyme, the surface tensions at the air-water interface derived by the drop volume method are given as a function of time up to 40 minutes in Figure 4. The most striking feature to be seen in Figure 4 is a faster change in, as well as a lowering of, the surface tension with increasing bulk concentration.

Lankveld and Lyklema (23) have also shown that the rate of decrease in interfacial tension is faster the higher the polymer concentration, for poly(vinyl-alcohol) adsorbed at the paraffin-oil/water interface. Adams et al. (10) deduced from their results with lysozyme films that low bulk concentration favours the formation of an adsorbed film containing a mixture of unfolded and native molecules, while a high concentration leads to the formation of a film of mostly native molecules.

In analogy with the earlier evaluation of the kinetics, γ - $t^{1/2}$ -curves and $\log \left(\frac{d\Pi}{dt} \right)$ - Π curves are presented in Figures 5 and 6, respectively, for all the concentrations. In Table II the parameters evaluated from the curves (cf. to Table I) are given for the different concentrations.

From the curves in Figures 5 and 6 it may be concluded that the effect of surface enlargement during the diffusion step becomes more evident, as the bulk protein concentration diminishes, being visible in Figure 6 for concentrations $\leq 0.01\%$ (w/w). When diffusion as a rate-determining step persists over longer times at lower concentrations, as seen for the quotient $\frac{\Delta t_{att}}{40}$ in Table II, the probability of the surface enlargement effect increases, as also suggested from the results in Table I. At the highest concentration of 1% (w/w) the step of penetration does not start until a surface pressure of about 13 mN m^{-1} , whereas at the lower concentrations the diffusion step lasts to a surface pressure of about 8 mN m^{-1} . This is in accordance with the lysozyme molecules spreading more easily at lower bulk concentrations during the diffusion step. The enhanced diffusion controlled adsorption to

the interface during the first 40 minutes by the concentration of 0.01% as compared to concentration of 0.1% can be due to the low flexibility of the lysozyme molecules at the interface. Compared at the same surface pressure, the value of ΔA_{ag} increases with decreasing concentration, which in combination with the reduced diffusion rate implies that the rate of interfacial tension decay is slowed down at lower concentrations, which is also observed in Figure 4.

List of symbols

- C_{pr} : protein concentration in molecules/l
- k_1 : rate constant for the adsorption of molecules at an interface
- $\bar{k}_1$: modified rate constant for the adsorption of active groups within a protein molecule at an interface
- n : number of adsorbed molecules per unit area
- n_{ag} : number of active groups adsorbed per unit area
- r : radius of the tip
- v_d : detached volume of a quickly formed drop
- v_m : maximum volume of a drop hanging below the orifice
- v_r : residual volume below the orifice after a rapidly formed drop has detached
- v_s : the volume extruded for the drop to be measured
- ΔA : mean surface area created in a film in order to adsorb a molecule
- ΔA_{ag} : mean surface area created in a film in order to adsorb an active group in the protein molecule
- v : number of active groups per protein molecule

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1. van den Tempel, M. Proc. 3rd Intern. Congr. Surface Activity, Cologne 1960, 2, 573-79.
2. Padday, J.F., in "Surface and Colloid Science" (E. Matijevic, Ed.), vol. 1, p. 39. Wiley-Interscience, New York, 1969.
3. Padday, J.F. and Russell, D.R., J. Coll. Sci. 15, 503 (1960).
4. Boucher, E.A., Grinchuk, T.M., and Zettlemoyer, A.L., J. Coll. I. Sci. 23, 600 (1967).
5. Addison, C.C. and Hutchinson, S.K., J. Chem. Soc. 3387 (1949).
6. Tornberg, E., J. Coll. I. Sci. 60, 50 (1977).
7. Boucher, E.A. and Evans, M.J.B., Proc. Roy Soc. A 346, 349 (1975).
8. MacRitchie, F. and Alexander, A.E., J. Coll. Sci., 18, 453 (1963).
9. Hamaguchi, K., J. Biochem., 31, 123 (1958).
10. Adams, D.J., Evans, M.T.A., Mitchell, J.R., Phillips, M.C., and Rees, P.M., J. Polymer Sci., 34, 167 (1971).
11. Graham, D.E. and Phillips, M.C., in "Theory and Practice of Emulsion Technology", p. 57 (A.L. Smith, Ed.). Symposium held at Brunel University, 16-18 Sept., 1974. Academic Press (1976).
12. Phillips, M.C., Evans, M.T.A., Graham, D.E. and Oldani, D., Colloid and Polymer Sci., 253, 424 (1975).
13. Yamashita, T. and Bull, H.B., J. Coll. I. Sci., 24, 30 (1967).
14. Graham, D.E., Thesis entitled "Structure of adsorbed protein films and stability of protein stabilised foams and emulsions". Unilever Research Laboratory, Colworth/Welwyn, The Frythe, Welwyn, Hertfordshire (1976).
15. Joos, P., Biochim. Biophys. Acta, 375, 1-9 (1975).
16. Evans, M.T.A., Mitchell, J.R., Musselwhite, P.R. and Irons, L., Advan. Exp. Med. Biol., 7, 122 (1970).
17. Muramatsu, M., and Ishii, T., Bull Chem. Soc., Japan, 43, 2364 (1970).
18. Harkins, W. and Brown, F.E., J. Am. Chem. Soc., 41, 499 (1919).
19. Hartland, S. and Srinivasan, P.S., J. Coll. I. Sci., 49, 318 (1974).
20. Pearson, F.W., and Whitaker, S., J. Coll. I. Sci., 54, 219 (1976).

21. Whitaker, S., J. Coll. I. Sci., 54, 231 (1976).
22. Wilkinson, M.C., J. Coll. I. Sci., 40, 14 (1972).
23. Lankveld, J.M.C., and Lyklema, J., J. Coll. I. Sci., 41, 454 (1972).
24. Frish, H.L. and Simha, R., J. Chem. Phys., 24, 652 (1956).
25. Frish, H.L. and Simha, R., J. Chem. Phys., 27, 702 (1957).
26. Graham, D.E., Personal communication.
27. Böhm, J.Th.C., Meded. Landbouwhogeschool Wageningen 74-5 (1974). Thesis.
28. Ward, A.F.H. and Tordai, L., J. Chem. Phys., 14, 453 (1946).
29. Ward, A.F.H., and Tordai, L., Recueil, 71, 572 (1952).
30. Davies, J.T., Biochem. Biophys. Acta, 11, 165 (1953).
31. Bull, H.B., J. Coll, I. Sci., 41, 305 (1972).

Table I. Parameters describing the kinetics of surface tension decay of lysozyme, β -lactoglobulin and BSA solutions of $10^{-2}\%$ (w/w) at the air/water interface measured with both the drop volume and the Wilhelmy plate methods.

Protein	Method of measurement	Rate-determining step	$\Pi_{att.}$		ΔA per ag (nm^2)	$\frac{\Delta \Pi_{att}}{\Pi_{40}}$ (%)	$\frac{\Delta t_{att}}{40}$ (%)
			$\Pi_t^{1/2}$ ($mN\ m^{-1}$)	Π_A			
Lysozyme	Drop volume	The effect of surface enlargement	2.1	2.0	-	17	18
		diffusion	11.2	10.5	0.2	72	58
		penetration	-	11.8	2.6	11	24
Lysozyme	Wilhelmy plate	diffusion	8.4	8.4	-	58	1
		penetration	-	11.3	2.4	20	10
		penetration	-	14.4	3.4	22	89
β -lactoglobulin	Drop volume	diffusion	12.8	11.0	0.4	55	1
		penetration	-	17.3	2.5	32	12
		penetration	-	18.0	10.6	3	14
		rearrangement	-	20.0	-	10	73
β -lactoglobulin	Wilhelmy plate	diffusion	13.6	13.4	-	68	0.3
		penetration	-	18.0	2.5	24	12.7
		penetration	-	19.6	6.7	8	87
BSA	Drop volume	diffusion	8.1	10.8	0.9	66	10
		penetration	-	16.3	2.0	34	90
BSA	Wilhelmy plate	diffusion	16.4	16.9	-	86	0.5
		penetration	-	18.8	5.4	10	8
		penetration	-	19.6	9.5	4	91.5

Table II. Parameters describing the kinetics of the lowering of surface tension of 1 M KCl solutions of lysozyme at the air-water interface measured with the drop volume method.

Conc. % (w/w)	Rate-determining step	$\Pi_{att.}$		ΔA per ag (nm ²)	$\frac{\Delta \Pi_{att}}{\Pi_{40}}$ (%)	$\frac{\Delta t_{att}}{40}$ (%)
		$\Pi_t^{1/2}$ (mN m ⁻¹)	Π_A			
1	diffusion	13.6	12.6	0.5	60	2
	penetration	-	19.1	2.1	31	17
	penetration	-	21.0	7.3	9	81
0.1	diffusion	8.6	6.7	0.4	37	7
	penetration	-	15.3	1.0	48	30
	penetration	-	18.0	5.2	15	63
0.01	diffusion	10.3	7.8	0.2	68	65
	penetration	-	11.5	0.7	32	35

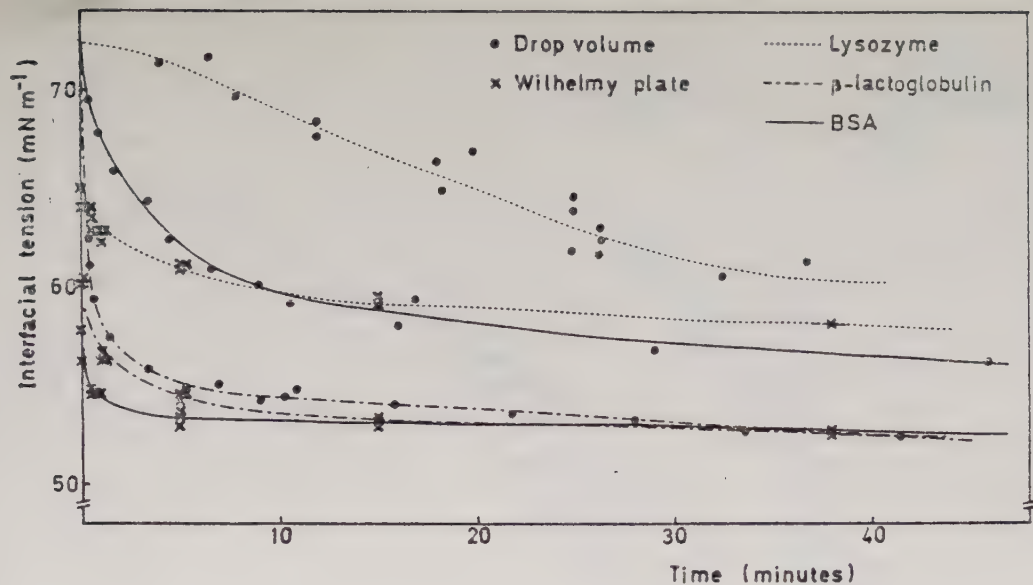


Figure 1. Time-dependence of interfacial tensions at the air/water interface for $10^{-2}\%$ (w/w) solutions of lysozyme, β -lactoglobulin and BSA measured with both the drop volume and the Wilhelmy plate methods.

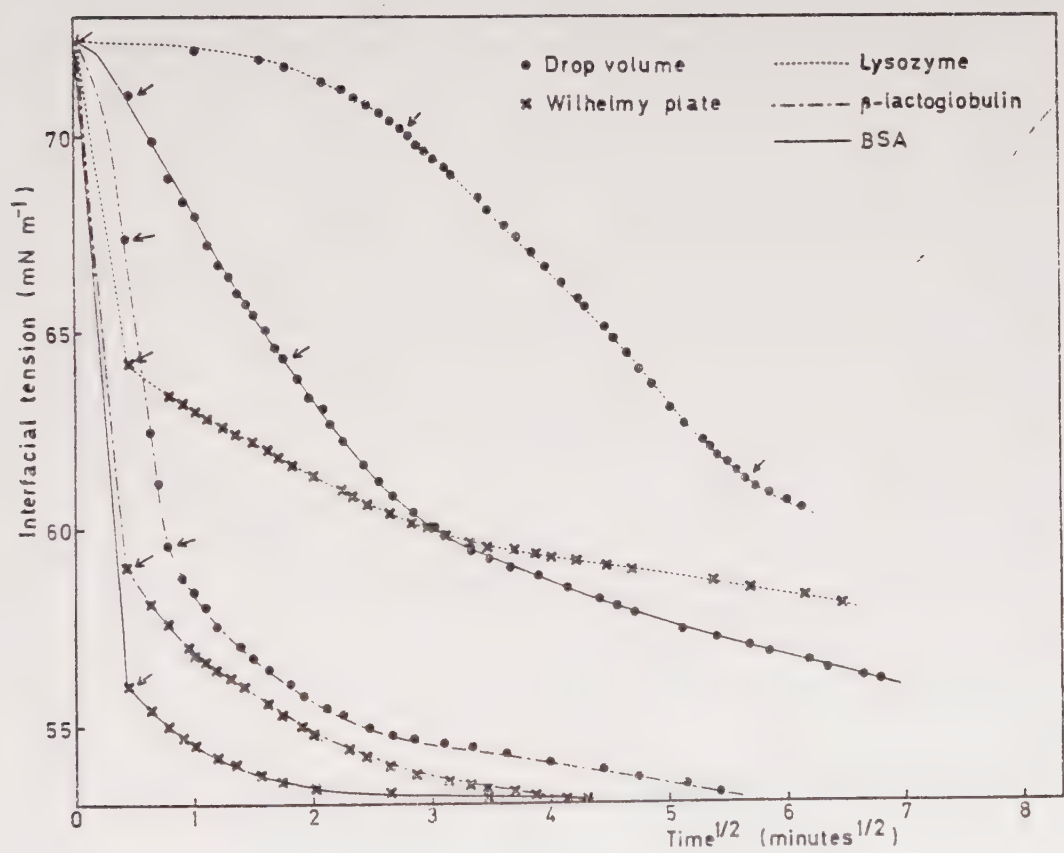


Figure 2. Interfacial tensions at the air/water interface as a function of $\text{time}^{1/2}$ for $10^{-2}\%$ (w/w) solutions of lysozyme, β -lactoglobulin and BSA measured with both the drop volume and the Wilhelmy plate methods.

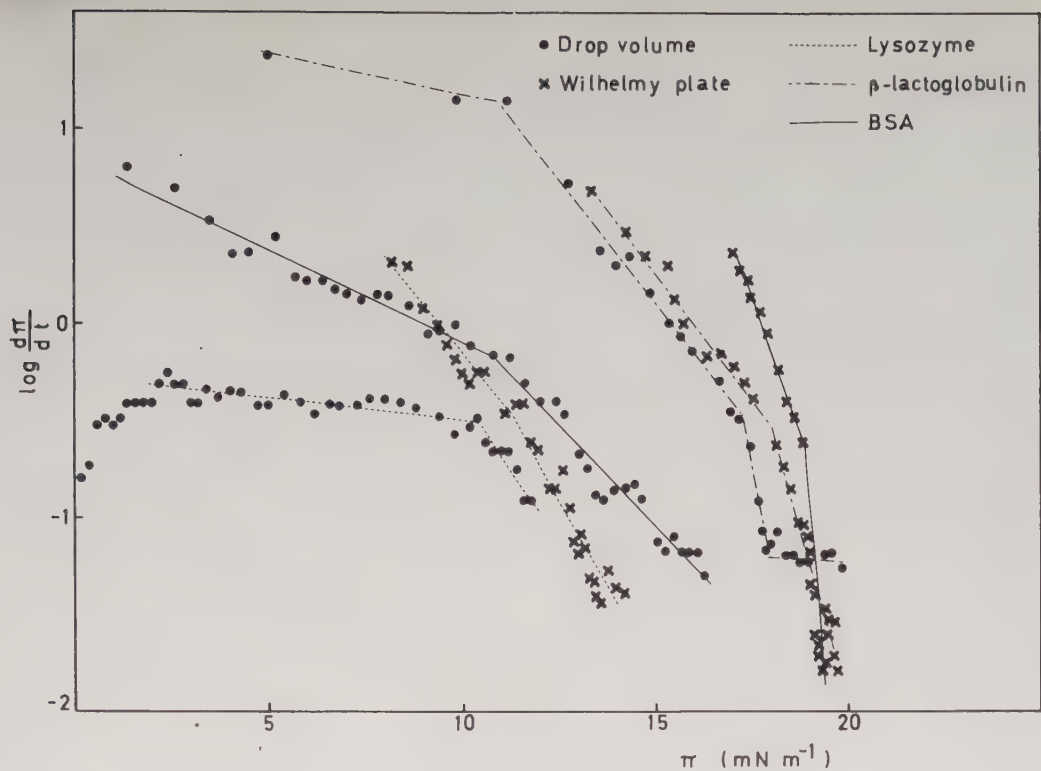


Figure 3. $\log \frac{d\Pi}{dt}$ as a function of Π for $10^{-2}\%$ (w/w) solutions of lysozyme, β -lactoglobulin and BSA adsorbing at the air/water interface and measured with both the drop volume and the Wilhelmy plate methods.

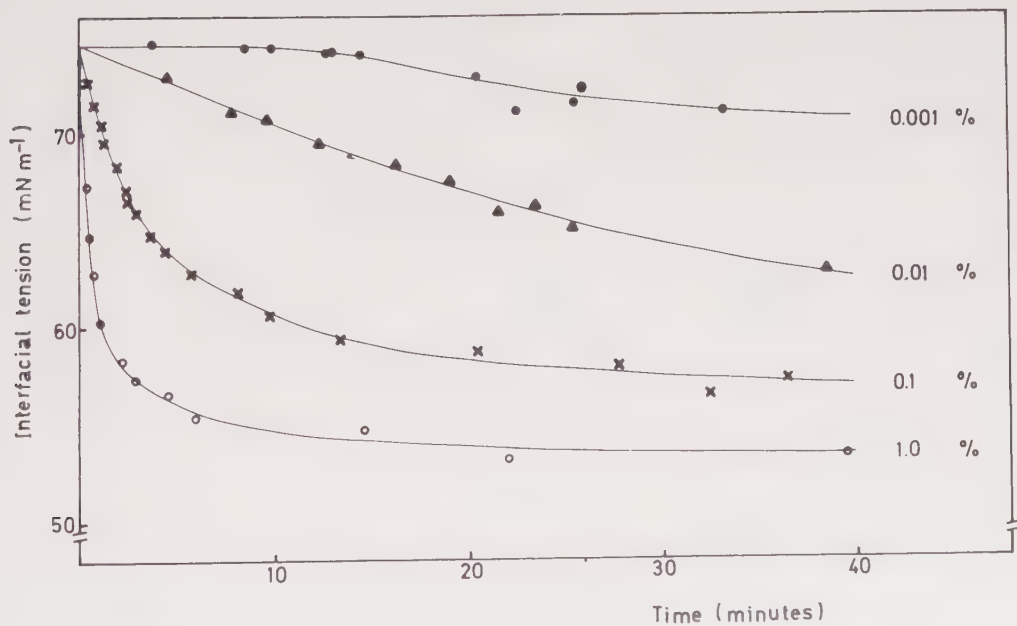


Figure 4. Time-dependence of interfacial tensions at the air/water interface for 1 M KCl solutions of lysozyme from 1.0 to 0.001% (w/w).

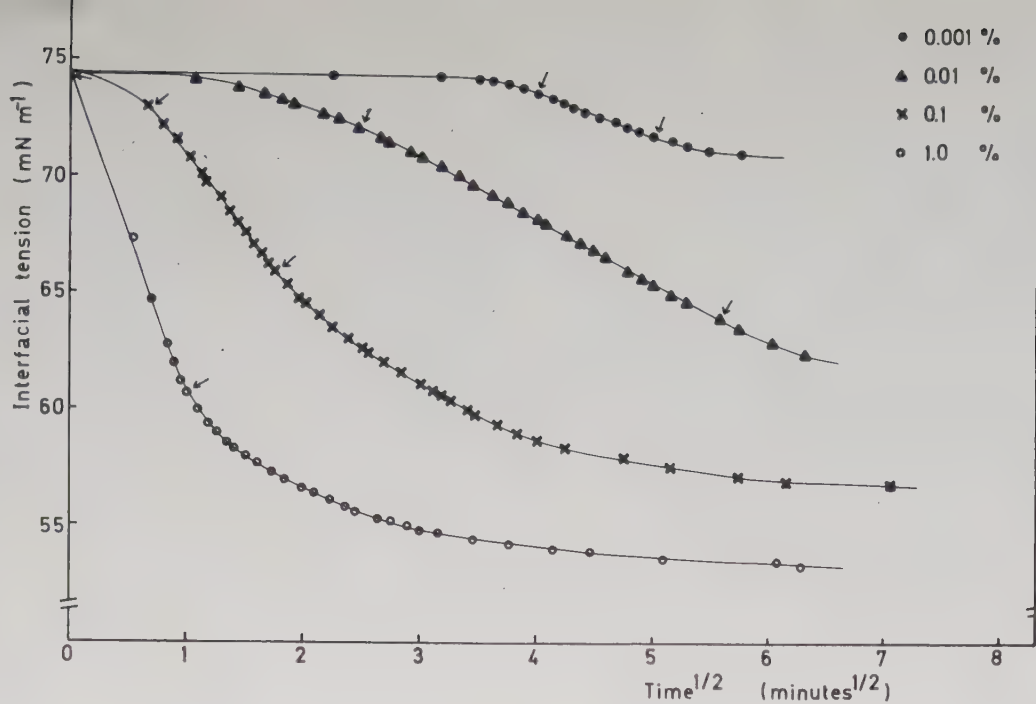


Figure 5. Interfacial tensions at the air/water interface as a function of time^{1/2} for 1 M KCl solutions of lysozyme from 1.0 to 0.001% (w/w).

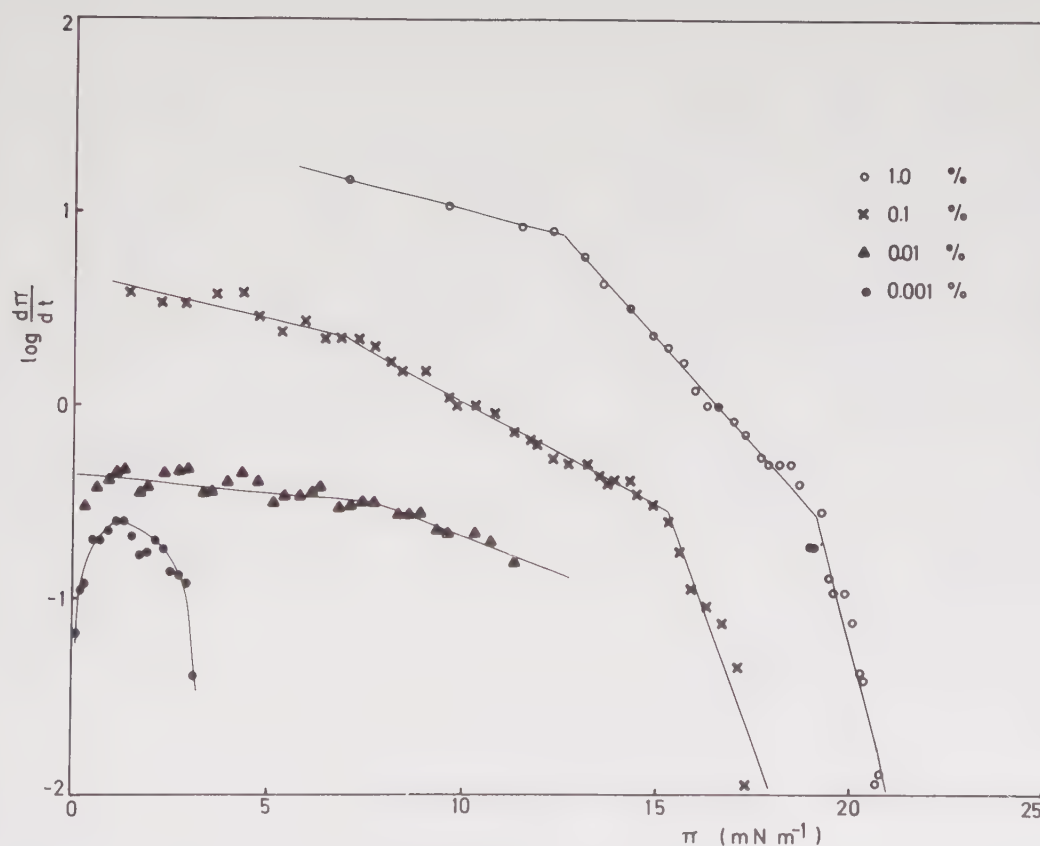


Figure 6. $\text{Log } \frac{d\pi}{dt}$ as a function of π for 1 M KCl solutions of lysozyme from 1.0 to 0.001% (w/w) adsorbing at the air/water interface.

THE INTERFACIAL BEHAVIOR OF THREE FOOD PROTEINS STUDIED BY THE DROP VOLUME TECHNIQUE

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Abstract

The adsorption behavior of three food proteins, a soy protein isolate, a sodium caseinate and a whey protein concentrate, at the air-water interface has been studied by the drop volume method. The kinetics of surface tension decay were evaluated in terms of different rate-determining steps at different ionic strengths and concentrations. This analysis indicates the following characteristics concerning the surface behavior of the protein systems studied.

The soy proteins diffuse slowly to the interface, probably due to the large particle size of the association complex of the soy proteins. Salt addition to 0.2 M NaCl speeds up the diffusion. The rate-determining step of penetration of molecules or segments into the film starts at a relatively low interfacial pressure, especially in 0.2 M NaCl, compared to the other proteins.

The whey proteins diffuse quickly to the interface, which is in accordance with their aqueous association; mainly small molecular complexes. Diffusion is slower and spreading easier in distilled water than in 0.2 M NaCl solution.

Although the caseinate has a complex quaternary structure, like the soy proteins, it has a very different surface behavior. The diffusion

step is rapid at concentrations above 10^{-3} wt. % and contributes to a large extent to the interfacial tension decay, especially when the caseinate is dispersed in 0.2 M NaCl solution. At a concentration of 10^{-3} wt. % and below, the rate of the diffusion step is drastically slowed down, with an accompanying drop in the surface activity of the protein. This type of surface behaviour can be explained if the migration of the caseinates to the interface takes place via the casein monomers in the bulk phase.

1. Introduction

In connection with the study of the emulsifying properties of proteins, their interfacial behaviour, as reflected in interfacial tension decay, is an important property. So far a considerable amount of work has been devoted to the study of spread protein films, which are formed by adding a known amount of protein directly to the water surface. However, the study of the adsorption of proteins at the interface from a subphase of known concentration is more relevant to what really happens during the emulsification process. There has only been one systematic study of the adsorption characteristics of a variety of proteins¹. The proteins studied contained very little or no complex quaternary structure, which is not the case for the protein systems available as emulsifiers in the food industry. The aim of this article is therefore to study the interfacial behaviour of such proteins, namely commercially available soy protein isolate, sodium caseinate and whey protein concentrate (WPC). The surface behaviour of the milk proteins has been studied previously. The high surface activity of the caseins has been elucidated by several authors¹⁻⁸, but the surface properties of the major whey proteins β -lactoglobulin^{4,9,10} and α -lactalbumin⁴ have been studied to a lesser extent.

In order to choose the appropriate technique to measure the interfacial tension decay of the proteins in relation to their emulsifying behaviour, a dynamic rather than an equilibrium technique was preferred. As has been shown and discussed in preceding papers (Tornberg and Hermansson¹¹; Tornberg¹²) the emulsification process

strongly influences the properties of protein-stabilized emulsions, and this further demonstrates the importance of the kinetic processes that exist during emulsification. In order to simulate the emulsification process, not only the kinetics of adsorption is of relevance, but also the concurrent extension of the surface, due to the continuously disturbed interface during emulsification. In a recent study¹⁰, a comparison has been made between the drop volume method and the Wilhelmy plate technique on the mode of adsorption of some different proteins at the air-water interface. The results showed different kinetics of adsorption for the two types of apparatus used, due to surface enlargement of the drop in the drop volume method throughout the process of surface tension decay. Moreover, the proteins investigated were to a different degree sensitive to this surface expansion. The interfacial tension as a function of time was therefore studied with an apparatus based on the drop volume technique.

2. Experimental

2.1 Materials

Soy protein isolate. A soy protein isolate produced under mild conditions was kindly provided by Central Soya. It was prepared from defatted soy bean flakes by extraction with deionized water, precipitation at pH 4.6, washing the curd with deionized water, neutralizing to pH 7.0 and spray drying. Analysis: protein (N x 6.25) 99.5% (dry weight); solubility determined according to Hermansson¹³ in distilled water and in 0.2 M sodium chloride solution at pH 7, denoted as (0 - 7) and (0.2 - 7), is 91.7% and 76.1%, respectively.

Caseinate. Spray blend caseinate (DMV, Holland), a commercially available sodium caseinate, was used. Analysis: protein (N x 6.43) 97.0% (dry weight); solubility determined as above in distilled water and in 0.2 M sodium chloride solution at pH 7, denoted as (0 - 7) and (0.2 - 7), is 97.4% and 95.4%, respectively.

Whey protein concentrate (WPC). The whey protein concentrate was obtained by ultra-filtration and spray drying of cheese whey. UF was carried out at 5°C in a pilot plant unit from De Danske Sukkerfabriker (DDS) with a membrane area of 2.2 m² and with a membrane type designated as 600 DDS. Analysis: protein (N x 6.55) 67.8% (dry weight); solubility determined as above in distilled water and in 0.2 M sodium chloride solution at pH 7, denoted as (0 - 7) and (0.2 - 7), is 100% and 98.2%, respectively.

2.2 Preparation of samples

Protein dispersions, based on the soluble protein content, in distilled water or sodium chloride solution were made with the Sorvall omni-mixer. The pH was adjusted with 0.2 M NaOH or 0.2 M HCl. To remove any protein particles that could sediment during the interfacial tension measurement, the dispersions were centrifuged for 30 minutes at 24000 x g and the supernatant was used. The water used was double-distilled¹⁴.

All glassware and stainless steel beakers used were washed first with sulfuric acid-dichromate solution and then with double-distilled water. All protein solutions were freshly made each day.

2.3 Interfacial tension measurements

Density measurements were carried out at 25°C for each protein solution using a pycnometer with a volume of 25 ml at 20°C.

The description of the surface tension apparatus and its mode of operation according to the drop volume principle are given elsewhere¹⁴. The procedure for making measurements with pure liquids and solutions that come to equilibrium quickly, as for the sodium chloride solutions, has been described in the same paper.

For measurements of time-dependent interfacial tensions, as in the case of proteins lowering the interfacial tension, a modified procedure is necessary. A drop of a certain volume, corresponding to a certain γ -value, is expelled rapidly, and the time necessary for the interfacial tension to fall to such a value that the drop becomes detached is measured. This procedure is repeated for differing drop sizes, i.e. for different values of the interfacial tension, and a plot of the interfacial tension as a function of time can be constructed¹⁰. The interfacial tension measurements were performed at the air-water interface, and the temperature was maintained at $25 \pm 0.1^\circ\text{C}$.

2.4 The kinetics of protein adsorption

The kinetics of protein adsorption at an interface in terms of different rate-determining steps is analyzed according to Ref. ¹⁰, and a summary of this analysis is given below.

The rate of reduction of interfacial tension is determined by three consecutive or concurrent processes^{4,16}: the diffusion of whole protein molecules or aggregates to and attachment at the interface; spreading or unfolding of already adsorbed molecules on the interface; molecular rearrangements and reconformations of adsorbed molecules.

Diffusion is considered to be the rate-determining step as long as a plot of the interfacial tension (γ) against $t^{1/2}$ is linear.

When a sufficiently high adsorption energy barrier exists, the diffusion will no longer be rate-determining, but the rate of protein penetration into the surface film will be rate-limiting. This barrier consists of the work done, $\Pi \Delta A_{ag}$, in creating an area ΔA_{ag} in a surface film of surface pressure Π in order to adsorb an active group (ag) in the protein molecule. The surface pressure, Π , is defined as $\Pi = \gamma_0 - \gamma$, where γ_0 is the initial interfacial tension. If a plot of $\ln \frac{d\Pi}{dt}$ versus Π is linear, the slope gives ΔA_{ag} .

When $\ln \frac{d\Pi}{dt}$ is independent of Π , i.e. when $\Delta A_{ag} \approx 0$, this is taken as an indication of a rearrangement process taking place, where no new areas, ΔA_{ag} , have to be created in order to adsorb protein segments. But the surface coverage is still enhanced, and thereby the surface tension lowered, by protein segments adsorbing at the surface unoccupied by protein segments in the preceding processes.

3. Results and discussion

3.1 Time-dependence of interfacial tensions for the three protein products at different ionic strengths and concentrations

The interfacial tension of the soy protein isolate, the WPC and the caseinate has been plotted as a function of time in figures 1 - 3. The initial interfacial tension (γ_0) was measured to be $71.91 \pm 0.16 \text{ mN m}^{-1}$ in distilled water, and equal to $72.25 \pm 0.17 \text{ mN m}^{-1}$ in 0.2 M NaCl solutions. It can be seen that the points are in general more scattered at lower concentrations, which is most obvious for the caseinate (0 - 7) dispersion.

The concentration-dependence of the interfacial tension can be more easily followed in figure 4, where the surface pressure attained after 40 minutes, $\pi_{40 \text{ minutes}}$, is plotted against the initial subphase concentration. Three regions are discernible in the figure: the high concentration range between 10^0 and 10^{-1} wt. %, the medium concentration range from 10^{-1} up to and including 10^{-3} wt. % and the low concentration range below 10^{-3} wt. %. At high concentrations, the surface activity of all the proteins under the different conditions is high and almost equal, whereas at medium concentrations the differences in surface behavior of the proteins become evident. The caseinate (0.2 - 7) system is most effective as an interfacial tension depresser and is more and less independent of concentration. The opposite behavior is observed for the soy proteins, which gradually lose their surface activity with decreasing subphase concentration. WPC (0 - 7) and caseinate (0 - 7) have a rather similar concentration-dependence in this range, and the curves are in between those of the caseinate (0.2 - 7) and the soy proteins. The addition of salt to the WPC-dispersions raises the surface activity of the WPC almost up to that of the caseinate (0.2 - 7). The increase in lowering of interfacial tension due to salt addition is also observed for the other two proteins. At very low concentrations, the caseinates drastically lose their surface activity and the soy proteins have no discernible interfacial depression effect, and thus the whey protein concentrates, which lose their surface activity less abruptly, are superior in this concentration region.

3.2 The evaluation of the kinetics of adsorption of the three protein systems

In order to evaluate the kinetics of adsorption of the proteins in terms of different rate-determining steps as discussed above, γ , from the fitted curves in figures 1, 2 and 3, has been plotted versus $t^{1/2}$. To exemplify the difference between the proteins at the same concentration (10^{-2} wt. %), γ has been plotted against $t^{1/2}$ in figure 5. The initial period before a linear relationship is established, which is most clearly visible for the soy protein, WPC (0 - 7) and caseinate (0 - 7) dispersions, can probably be attributed to the enlargement of the surface of the drop being faster than the build up of an adsorption barrier; both these phenomena being a consequence of the surface tension decay performed by the adsorbed molecules.¹⁰ It is further confirmed by $\frac{d\Pi}{dt}$, which increases during this initial period as a function of Π . This is shown by results for the soy proteins, the WPC (0 - 7) and the caseinate (0 - 7) at the same concentration of 10^{-2} wt. % in figure 6, where $\log \frac{d\Pi}{dt}$ is plotted as a function of Π . The maximum slope, which corresponds to the linear part of the plots marked with two arrows given in figure 5, is always taken for diffusion being the rate-determining process. After the linear part of the $\gamma - t^{1/2}$ curve, the rate of decrease in surface tension with increasing $t^{1/2}$ falls off.

$\log \frac{d\Pi}{dt}$ has been plotted against Π in figure 6 for the three proteins dispersed in distilled water or in sodium chloride solution at a concentration of 10^{-2} (w/w) %. The slopes were obtained by computer differentiation of the fitted curves in figures 1, 2 and 3, which were approximated by parabolas for small sections. Linear parts derived by linear regression analysis were obtained with evident breaking points for all the protein dispersions, as seen in figure 6. The

correlation coefficients for the linear parts obtained could vary from 0.68 to 0.99. The same type of graphs as in figures 5 and 6 are shown in figures 7 and 8 for the soy protein (0 - 7) dispersion at different concentrations as an example of the concentration dependence.

To summarize the quantitative evaluation of the typical graphs from figures 5 to 8, the surface pressure attained (π_{att}) and ΔA_{ag} obtained during the different rate-determining steps are collected in tables. In these tables also two quotients $\frac{\Delta t_{att}}{40}$ and $\frac{\Delta \pi_{att}}{\pi_{40}}$ are given, which indicate, respectively, the relative contribution of the time elapsed and π_{att} of each rate-determining step in relation to 40 minutes and to the surface pressure attained after 40 minutes. In tables 1 to 3 the parameters describing the kinetics of lowering the interfacial tension have been collected for the soy proteins, the WPC and the caseinates at different subphase concentrations and ionic strengths.

From table 1, representing the soy proteins, it can be seen that the surface pressures attained during the "diffusion" steps, denoted as $\pi_t 1/2$, evaluated in the case of soy protein (0 - 7) from figure 7, and the surface pressures for the first steps derived from figure 8, in the case of soy protein (0 - 7), are the same within the limits of error. This implies that it is possible to distinguish when the diffusion or the step of penetration of protein segments into the surface film is the rate-controlling factor. The analysis of the kinetics of adsorption that follows is based on this assumption (cf. Ref.¹⁰). The step of penetration involves a higher number of segments participating in the process as compared to the diffusion step, which is reflected in the greater

ΔA_{ag} values given in the table. The value of ΔA_{ag} also grows with the surface pressure, probably due to an increased structure in the film with increasing surface pressure, inducing more protein segments to participate in the rearrangement processes, as discussed in an earlier paper¹⁰.

The effect of surface enlargement becomes evident only at a concentration as low as $3 \cdot 10^{-2}$ wt. % for the soy protein (0 - 7) and the soy protein (0.2 - 7). This behavior persists over longer periods of time in the case of the soy protein dispersed in distilled water than in sodium chloride solution (compare the quotients

$\frac{\Delta t_{att}}{40}$). Comparison of the soy proteins at different ionic strengths

further confirms that the decline of γ by soy protein (0 - 7) is more diffusion controlled, especially at lower concentrations, and

that the diffusion is slower, as elucidated from the quotients

$\frac{\Delta t_{att}}{40}$. A relationship between slow diffusion and the appearance of the effect of surface enlargement has earlier been established¹⁰.

The faster start of the penetration step at a lower Π for soy protein (0.2 - 7) than for soy protein (0 - 7) at concentrations below 1% (w/w), can be attributed to the soy protein (0.2 - 7) spreading more easily during the diffusion step. At the same surface pressure the values of ΔA_{ag} during the penetration steps are larger with the addition of salt, which indicates slower rearrangement processes taking place in the surface film of soy protein (0.2 - 7) (cf. ref.¹⁰). This will lead to a slower surface tension decay for soy protein (0.2 - 7) than for soy protein (0 - 7) during the last stages of the surface depression process, which can also be observed in figure 1. The step of rearrangement with $\Delta A_{ag} \approx 0$ is only visible for the soy protein (0.2 - 7) in the time range studied.

The results in table 1 show that at lower bulk concentrations the penetration controlled step starts at an earlier stage in the adsorption process, i.e. at a lower Π , and at the same surface pressure the values of ΔA_{ag} increase with decreasing concentration. The diffusion-controlled adsorption is also slower with decreasing concentration. This gives at first the possibility for the soy proteins to spread more easily at low bulk concentrations during the diffusion step, thereby providing an adsorption barrier against further adsorption at a lower Π . Secondly, the interfacial tension decay is slowed down at lower concentrations, due to both the reduced diffusion rate and the enhanced number of residues (larger ΔA_{ag}) participating in the rearrangement processes during the penetration-controlled steps compared at the same Π .

The results of the evaluation of the kinetics of adsorption of WPC can be seen in table 2. From a concentration of 10^{-1} to 10^{-3} wt. %, the same tendency as for the soy proteins can be seen for both WPC-dispersions, i.e. diffusion is slower, the penetration step starts at a lower Π and ΔA_{ag} is larger at the same Π for decreasing concentration. The effect of surface enlargement is visible at a concentration of 10^{-2} wt. % for the WPC (0 - 7) and occurs at $1 \cdot 10^{-3}$ and $6 \cdot 10^{-4}$ wt. % for both the WPC (0 - 7) and the WPC (0.2 - 7) dispersions, respectively. This step is slower for the WPC (0 - 7) dispersions, which is most evident at the low concentration of $6 \cdot 10^{-4}$ wt. %. At a concentration of 10^{-1} wt. % the diffusion-controlled step persists to a higher Π by addition of salt, which indicates that spreading during the rate-determining step of diffusion is more facilitated in distilled water. When the concentration is lowered to 10^{-2} wt. %, it can be seen for the WPC (0 - 7) that the energy barriers against adsorption for the diffusion and the first penetration step are indistinguishable within the limits

of error. But still the same relationships between the two ionic strengths are valid, i.e. the surface is more covered by diffusion from the bulk with addition of salt, and spreading is favoured in distilled water, which is also the case at the two lowest concentrations of $1 \cdot 10^{-3}$ and $6 \cdot 10^{-4}$ wt. %.

The parameters describing the kinetics of caseinate adsorption are collected in table 3. Similar phenomena, concerning the concentration-dependence, are obtained with caseinate as have been observed with WPC and soy protein dispersions, i.e. the diffusion step is faster and persists to a greater surface pressure at higher concentrations, and therefore spreading is favoured during the diffusion-controlled step at lower concentrations. When the caseinate is dispersed in distilled water the effect of surface enlargement occurs already at a concentration of 10^{-2} wt. %, whereas this step is not discernible until a concentration of 10^{-3} for the caseinate (0.2 - 7). The effect of surface enlargement for the caseinate (0.2 - 7) does not reach to such a high surface pressure as in the case of the caseinate (0 - 7) dispersion. It is interesting to note with the caseinate, that when the subphase concentration is decreased from 10^{-2} wt. % to 10^{-3} wt. %, the diffusion step is significantly slowed down, as reflected in a drastic increase of $\frac{\Delta t_{att}}{40}$. This is especially pronounced for the caseinate (0 - 7), where the step of diffusion, that precedes the effect of surface enlargement, is that slow to be detected. However, this evaluation should be taken with some caution because the scatter in the results is rather wide for caseinate (0 - 7) at the concentration of 10^{-3} wt. % (see figure 3). A comparison of the caseinate system, with and without salt addition shows that at a concentration of 10^{-1} wt. %, the surface behaviors are rather similar. However, at the lower concentrations the caseinate (0.2 - 7) system is governed more by diffusion, when the interfacial

tension is lowered, than is the caseinate (0 - 7) system. Furthermore, the diffusion is slower for caseinate (0 - 7) than for caseinate (0.2 - 7).

3.3 Comparison of surface behavior of the three proteins

From tables 1, 2 and 3 a comparison between the proteins at different ionic strengths can be made at the concentration level of 10^{-1} % (w/w). The proteins can effectively be divided into two groups. The first group consists of the soy protein dispersed in both distilled water and 0.2 M NaCl solution and the WPC (0 - 7) dispersion, in which all the films start the penetration-controlled step at a relatively low surface pressure around 10 mN m^{-1} . The second group of both the caseinates and the WPC (0.2 - 7) system has a diffusion-controlled adsorption up to $\Pi \approx 17 \text{ mN m}^{-1}$. It is also already obvious at this high concentration that the step of diffusion is slower for the soy proteins, especially for the soy protein (0 - 7), compared to the other protein systems. Within the first group the soy protein (0.2 - 7) is probably most easily spread, having the diffusion-determined step up to the lowest Π . According to this reasoning the WPC (0 - 7) system comes next in spreading ability, closely followed by the soy protein (0 - 7) product. In the next group, where diffusion-control is the dominating rate-determining step during the interfacial tension decay at this concentration, the quotient $\frac{\Delta\Pi_{\text{att}}}{\Pi_{40}}$ for this step is 73, 68 and 66% for caseinate (0.2 - 7), caseinate (0 - 7) and WPC (0.2 - 7), respectively.

At a lower concentration (10^{-2} wt. %) the empirical division into two groups still holds, but there is now more divergence in surface behavior of the proteins within the groups. Within the first group at the concentration 10^{-2} wt. % the effect of surface enlargement occurs for each protein. This step reaches the highest

surface pressure and endures over the longest period of time for the soy protein (0 - 7), followed by soy protein (0.2 - 7) and thereafter the fastest acting WPC (0 - 7). The same order within these proteins is even more evident, concerning the slowness of the subsequent, i.e. the diffusion-controlled step. The spreading of the soy protein (0.2 - 7) is still most favoured among these proteins, as the first penetration step starts at the lowest Π .

In the next group, the effect of surface enlargement is only visible for the caseinate (0 - 7), and this step is almost as slow as for the soy protein (0.2 - 7) system. The diffusion step is equally fast for the WPC (0.2 - 7), the caseinate (0.2 - 7) and the caseinate (0 - 7) systems, but reaches a much higher surface pressure in the case of caseinate (0.2 - 7) followed by WPC (0.2 - 7), which is now at this concentration more diffusion-controlled than the caseinate (0 - 7).

At the low concentration of 10^{-3} wt. %, the surface activity of the soy proteins is too low to be evaluated with any accuracy. The effect of surface enlargement is visible for both the caseinate and WPC systems at the different ionic strengths, being slower and reaching a higher surface pressure in the case of the caseinates. The diffusion step is still most pronounced for the caseinate (0.2 - 7) product, contributing as much as 67% to the interfacial tension depression during the first 40 minutes, compared to caseinate (0 - 7) and the whey protein concentrates, with values of 44 and ca. 28%, respectively. The diffusion of the caseinates is also much slower than that of the whey protein concentrates, which can be judged from the time contribution of this step during the first 40 minutes. The penetration-controlled step starts at an earlier stage during the adsorption process for the whey protein concentrates than for the caseinates.

3.4 Correlation between bulk and interfacial properties of the protein systems

In order to correlate the structural properties of the proteins in the bulk with those at the interface, a short summary of the type of surface films that can be formed and factors that govern this formation is given below.

According to Evans et al.⁵ surface films of proteins can roughly be divided into two extremes, dilute films consisting of unfolded molecules and concentrated films, where most of the native structure of the protein is left within the surface film. The work of Malcolm¹⁵ suggests that even in dilute films, proteins and polypeptides may maintain their secondary structure. If this is true, surface denaturation of the protein systems studied in this work will only involve different degrees of the destruction of the quaternary and tertiary structure of the protein products. The findings of Evans et al.⁵ have indicated that strongly aggregated proteins are more likely to form concentrated films than monomeric species, and they also concluded that a protein with a lack of well-defined tertiary structure adsorbs faster to an interface, unfolds more easily, and causes a larger change in interfacial tension.

The process of diffusion of the proteins to the interface can be considered to be mainly governed by the diffusion constant (D) of the protein molecule and by the probability (Z) of resulting adsorption, when the protein molecules collide with the interface. The diffusion constant depends on the molecular or particle size of the protein. The probability of a molecular collision with the interface resulting in adsorption (Z) will probably depend on the degree of electrostatic hindrance during adsorption and on the hydrophobicity of the molecules.

Addition of monovalent salts, as in the case of sodium chloride, should be expected to suppress the electrical double layer surrounding the protein colloid, and therefore to decrease the electrostatic repulsion during adsorption and thereby increasing Z. MacRitchie and Alexander¹⁶ have shown that the net rate of collision of protein molecules with the surface depends upon pH, being a maximum near the isoelectric point and decreasing on either side. Increasing tendency to unfold at interfaces with increasing hydrophobicity of the proteins has been observed by Birdi¹⁷ for the adsorption of several proteins at the air-water interface.

Soy proteins have a complex quaternary structure in bulk. The two major soy protein fractions, the 7S and 11S globulins, have a particle weight of 180 - 210 000¹⁸ and 309 - 363 000¹⁹, respectively. The 7S globulin consists of at least 9 subunits (molecular weight of 22 000 - 24 000)¹⁸, and the 11S globulin consists of six acidic subunits (molecular weight of 35 000) and six basic subunits each having a molecular weight of 20 000¹⁹. The tertiary structures of the two major soy proteins appear to be compact structures stabilized by hydrophobic bonds²⁰, but this folded structure has been proved to be easily destroyed upon dissociation of the proteins into subunits²². According to Wolf²⁰ and Koshiyama²¹ association and aggregation of the 7S and 11S globulins is favoured at 0.1 - 0.2 M NaCl.

The values of the quotient $\frac{\Delta t_{att}}{40}$ for the surface enlargement and the diffusion step is large for the soy proteins in comparison with the two other proteins, when compared at the same bulk concentration, which indicates that the soy proteins migrate to the interface with the gross quaternary structure intact. From table 1 it is clear that the adsorption of soy protein (0 - 7) at an interface is more diffusion controlled, and that this step persists for longer periods of time than for soy protein (0.2 - 7). This may seem to be contradictory

because the soy protein is aggregated more in 0.2 M NaCl solution than in distilled water. The anomaly can be explained, firstly, by a greater Z-value in the case of the soy protein (0.2 - 7), due to reduced electrostatic repulsion during adsorption, and secondly, by the increase in spreading ability of the soy protein (0.2 - 7), as revealed from the results in table 1.

Contrary to the soy proteins, the whey proteins do not form molecular complexes with high aggregation numbers. A whey protein concentrate contains mainly β -lactoglobulin and α -lactalbumin. β -Lactoglobulin has a molecular weight of 18 400 and makes up 59% of the whey proteins, and the monomer is associated to form dimers at room temperature in solutions between pH 3 and 7²³. α -Lactalbumin with a molecular weight of 14 200 makes up 22% of the whey proteins, and between pH 5.4 and 9.0 α -lactalbumin has a rather stable conformation and appears in a monomeric form²³.

At protein concentrations of 10^{-2} and 10^{-3} wt. % both the steps of surface enlargement and diffusion for the whey proteins have relatively short duration when compared with the other proteins, especially the soy proteins, which is in accordance with the whey proteins being small molecules. Without salt the diffusion to the interface by the WPC dispersions is somewhat slower, and is most evident in the observation that the effect of surface enlargement occurs at a concentration of 10^{-2} wt. % and lasts over longer periods of time at the lower concentrations. The probability of the WPC (0.2 - 7) to adsorb on collision with the interface is higher than that of WPC (0 - 7), which evidently makes the diffusion process faster. Clearly visible in table 2 is that the WPC (0.2 - 7) covers the interface to a higher degree by diffusion, at every concentration, than the whey protein concentrate dispersed in distilled water. This is probably an effect

of the WPC (0 - 7) spreading more easily at the interface, which is seen from table 2, where the step of penetration starts at lower Π .

The native casein 'micelle' subunit is considered to have a particle weight of approximately 250 000, each subunit containing about 12 molecules of mainly α_s -casein, β -casein and κ -casein²⁴. The molecular weights of the caseins vary from 24 000 down to 19 000. Schmidt and Buchheim²⁵ suggest that the preparation of sodium caseinate destroys the original properties of the casein 'micelle' and possibly also the structure of the submicelles. Some other observations made by Creamer and Berry²⁶ have shown, that complete disaggregation of the caseins did not alter their subsequent ability to form subunits, which were indistinguishable from those obtained directly from casein micelles by gel filtration.

On comparison of the proteins at a concentration of 10^{-1} and 10^{-2} wt. % it can be seen that the duration of the diffusion step of the caseinates is of the same magnitude as for the whey proteins, the rate of diffusion of the soy proteins being much slower. Although the particle weight of the casein micelle subunit is the same order of magnitude as for the 7S globulin, the diffusion is accomplished as fast as in the case of the WPC. This can be explained if the migration to the interface of the caseinates is performed mainly by the free casein molecules, which are in equilibrium with those in the submicelles. The results obtained from the work by Creamer and Berry²⁶ show that the casein 'micelle' subunits were found to be in equilibrium with their component caseins. In a later article of Creamer et al.²⁷, they have shown results consistent with a model in which β -casein in the interior of the submicelle can exchange with that on the surface and this, in turn, can exchange with that

in the milk serum. These observations might explain the abrupt increase in the quotient $\frac{\Delta t_{att}}{40}$ for the diffusion step at the concentration of 10^{-3} wt. %, by assuming that the migration of the β -casein molecules from the interior of the submicelle is the rate-controlling factor at this low concentration.

The curve of caseinate (0.2 - 7) in figure 4 seems to be analogous to that of an amphiphilic substance with a critical micelle concentration (c.m.c.) just below 10^{-3} wt. %. Payens and Schmidt²⁴ have in fact been able to demonstrate the existence of a c.m.c. around $4 \cdot 10^{-2}$ wt. % for the association of β -casein molecules at pH 7 and an ionic strength of 0.2. The lower c.m.c. formation of the submicelles, as revealed by these measurements, compared to that of β -casein, could be attributed to the more stable complex formation between the different casein monomers than between themselves, as observed by Creamer and Berry²⁶.

The diffusion controlled occupation of the interface is very evident for the caseinates, especially at an ionic strength of 0.2, compared to the other proteins. These results indicate that the very flexible, random coil-like casein molecules have simpler kinetics than the other proteins, involving diffusion to the interface and direct anchoring of the freely available hydrophobic segments. Due to the higher electrostatic repulsion between adsorbed species at the interface for caseinate (0 - 7) compared to caseinate (0.2 - 7), the caseinate (0.2 - 7) molecules can more densely pack at the interface. This will give rise to an adsorption barrier at a higher Π for caseinate (0.2 - 7), which also has been observed. The observed differences between the caseinate dispersed in distilled water and

in 0.2 M NaCl solution is that the former diffuses more slowly to the interface, which results in the effect of surface enlargement being visible at a higher concentration. The reduced speed of the diffusion process of the caseinate (0 - 7) might be explained by an enhanced electrostatic repulsion during adsorption, and thereby a reduction of Z.

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References

1. Graham, D.E. 1976, Thesis entitled "Structure of adsorbed protein films and stability of protein stabilised foams and emulsions".
Unilever Research Laboratory, Colworth/Welwyn, The Frythe, Welwyn, Hertfordshire.
2. Walker, D.A. Third Int. Congress of Surface Activity 1960,
4 (41) 351.
3. Musselwhite, P.R. J. Coll. Interface Sci. 1964, 21, 99.
4. Mitchell, J; Irons, L; Palmer, G.J. Biochem. Biophys. Acta
1970, 200, 138.
5. Evans, M.T.A.; Mitchell, J; Musselwhite, P.R.; Irons, L.
In "Advances in Experimental Medicine and Biology" (Blank, M.,
ed.), 1970, vol. 7, p. 1 - 20, Plenum Press, New York.
6. Benjamins, J.; Feijter, J.A.; Evans, M.T.A.; Graham, D.E.;
Phillips, M.C. Faraday Disc. of Chem. Soc. 1973, 59, 218.
7. Mita, T.; Yamada, K.; Matsumoto, S.; Yonezawa, D. J. Texture
Stud. 1973, 4, 41.
8. Phillips, M.C.; Evans, M.T.A.; Graham, D.E.; Oldani, D.
Colloid & Polymer Sci. 1975, 253, 424.
9. Joos, P. Biochem. Biophys. Acta 1975, 375, 1.
10. Tornberg, E. J. Coll. Interface Sci. In press (1978).
11. Tornberg, E.; Hermansson, A.M. J. Food Sci. 1977, 42, 468.
12. Tornberg, E. Submitted for publication in J. Food Sci.
13. Hermansson, A.M. Functional Properties of Proteins for Foods -
Solubility. AULs halvårsskrift No. 2, Kemicentrum, Lund, Sweden.
1973.
14. Tornberg, E. J. Coll. Interface Sci. 1977, 60, 50.
15. Malcolm, B.R. J. Polymer Sci. 1971, 34, 87.
16. MacRitchie, F.; Alexander, A.E. J. Coll. Interface Sci.
1963, 18, 464.

17. Birdi, K.S. J. Coll. Interface Sci. 1973, 43, 545.
18. Koshiyama, I. Agr. Biol. Chem. 1971, 35, 385.
19. Badley, R.A.; Atkinson, D.; Hauser, H.; Oldani, D.; Green, J.P.; Stubbs, J.M. Biochem. Biophys. Acta 1975, 412, 214.
20. Wolf, W.J. In "Soybeans: Chemistry and Technology" (Smith, A.K.; Circle, S.J., eds.), 1972, p. 93, AVI Publishing Company Inc., Westport, Connecticut.
21. Koshiyama, I. Agr. Biol. Chem. 1968, 32, 879.
22. Koshiyama, I. J. Sci. Fd Agric. 1972, 23, 853.
23. Lyster, R.L.J. J. Dairy Res. 1972, 39, 279.
24. Schmidt, D.G.; Payens, T.A.J. Surface and Colloid Sci. 1976, 9, 165.
25. Schmidt, D.G.; Buchheim, W. Neth. Milk Dairy J. 1975, 29, 17.
26. Creamer, L.K.; Berry, G.D. J. Dairy Res. 1975, 42, 169.
27. Creamer, L.K.; Berry, G.D.; Mills, O.E. N.Z.J. Dairy Sci. Technol. 1977, 12, 58.

Table 1. Parameters describing the kinetics of the interfacial tension decay at the air-water interface of soy protein dispersions at different ionic strengths and concentrations.

Type of protein system	Conc. % (w/w)	Rate-determining step	$\Pi_{att.}$		ΔA per ag (nm^2)	$\frac{\Delta t_{att.}}{40}$ (%)	$\frac{\Delta \Pi_{att.}}{\Pi_{40}}$ (%)
			$\Pi_t^{1/2}$ ($mN\ m^{-1}$)	Π_A			
Soy protein (0.2 - 7)	10^0	Diffusion	15.6	17.4	-	1	63
		Penetration	-	25.1	2.6	57	33
		Rearrangement	-	26.2	-	42	3
Soy protein (0.2 - 7)	10^{-1}	Diffusion	9.5	8.5	-	2	43
		Penetration	-	20.3	1.7	68	54
		Penetration	-	20.8	7.0	30	3
Soy protein (0.2 - 7)	$3 \cdot 10^{-2}$	The effect of surface enlargement	1.7	1.7	-	2	10
		Diffusion	7.0	6.4	0.7	3	31
		Penetration	-	15.6	1.4	64	55
		Penetration	-	16.3	6.5	31	4
Soy protein (0.2 - 7)	$1 \cdot 10^{-2}$	The effect of surface enlargement	0.9	0.5	-	4	5
		Diffusion	6.4	5.8	0.2	12	41
		Penetration	-	10.7	1.7	38	35
		Rearrangement	-	13.2	-	46	19
Soy protein (0 - 7)	10^0	Diffusion	15.5	15.3	0.5	2	58
		Penetration	-	25.4	1.6	37	37
		Penetration	-	26.7	6.1	61	5
Soy protein (0 - 7)	10^{-1}	Diffusion	12.6	11.6	0.3	8	55
		Penetration	-	22.1	1.3	92	45
Soy protein (0 - 7)	$3 \cdot 10^{-2}$	The effect of surface enlargement	1.9	2.5	-	10	14
		Diffusion	9.5	8.9	0.3	16	44
		Penetration	-	15.8	1.4	74	42
Soy protein (0 - 7)	$1 \cdot 10^{-2}$	The effect of surface enlargement	1.9	2.9	-	24	24
		Diffusion	9.9	10.0	0.6	76	76

Table 2. Parameters describing the kinetics of the lowering of interfacial tension at the air-water interface of WPC dispersions at different ionic strengths and concentrations.

Type of protein system	Conc. % (w/w)	Rate-determining step	$\Pi_{att.}$		ΔA per ag (nm^2)	$\frac{\Delta t_{att.}}{40} (\%)$	$\frac{\Delta \Pi_{att.}}{\Pi_{40}} (\%)$
			$\Pi_t^{1/2}$ (mN m^{-1})	Π_A			
C (0.2 - 7)	10^{-1}	Diffusion	15.7	16.7	-	1	66
		Penetration	-	24.0	2.6	68	31
		Rearrangement	-	24.8	-	31	3
C (0.2 - 7)	10^{-2}	Diffusion	13.3	14.0	1.0	2	61
		Penetration	-	22.1	2.3	67	38
		Penetration	-	22.4	12.1	31	1
C (0.2 - 7)	$1 \cdot 10^{-3}$	The effect of surface enlargement	1.3	2.3	-	5	12
		Diffusion	4.9	7.3	1.1	9	28
		Penetration	-	14.1	0.4	60	52
		Penetration	-	15.5	2.7	26	8
C (0.2 - 7)	$6 \cdot 10^{-4}$	The effect of surface enlargement	1.5	1.2	-	13	15
		Diffusion	8.1	10.5	0.5	87	85
C (0 - 7)	10^{-1}	Diffusion	9.9	11.1	0.6	1	47
		Penetration	-	20.9	1.9	43	47
		Penetration	-	22.0	6.8	56	6
C (0 - 7)	$1 \cdot 10^{-2}$	The effect of surface enlargement	1.1	3.6	-	1	13
		Diffusion and penetration	8.1	17.6	1.1	3 and 33	30 and 51
		Penetration	-	18.8	16.2	63	6
C (0 - 7)	10^{-3}	The effect of surface enlargement	1.0	1.3	-	5	10
		Diffusion	5.3	4.7	0.1	5	27
		Penetration	-	10.4	1.2	27	48
		Penetration	-	12.2	4.8	63	15
C (0 - 7)	$6 \cdot 10^{-4}$	The effect of surface enlargement	2.6	1.2	-	25	23
		Diffusion	7.8	6.9	0.1	54	65
		Penetration	-	8.2	3.0	16	10
		Penetration	-	8.4	24.3	5	2

Table 3. Parameters describing the kinetics of the interfacial tension decay at the air-water interface of caseinate dispersions at different ionic strengths and concentrations.

Type of protein system	Conc. % (w/w)	Rate-determining step	Π_{att}		ΔA per ag (nm^2)	$\frac{\Delta t_{att.}}{40}$ %	$\frac{\Delta \Pi_{att.}}{\Pi_{40}}$ (%)
			$\Pi_t^{1/2}$	Π_A			
			(mN m^{-1})				
Caseinate (0.2 - 7)	10^{-1}	Diffusion	17.1	18.2	1.0	1	73
		Penetration	-	23.5	2.7	13	24
		Penetration	-	24.3	15.6	86	3
Caseinate (0.2 - 7)	10^{-2}	Diffusion	17.5	16.1	0.4	2	72
		Penetration	-	22.8	2.3	16	26
		Penetration	-	23.3	20.1	82	2
Caseinate (0.2 - 7)	10^{-3}	The effect of surface enlargement	2.0	2.3	-	11	13
		Diffusion	11.2	15.7	0.6	31	67
		Penetration	-	16.8	4.9	58	20
Caseinate (0.2 - 7)	$6 \cdot 10^{-4}$	The effect of surface enlargement	0.4	0.9	-	19	10
		Diffusion	6.1	6.3	0.5	52	77
		Penetration	-	7.1	5.8	29	13
Caseinate (0 - 7)	10^{-1}	Diffusion	18.1	16.2	0.5	1	68
		Penetration	-	23.0	2.5	13	23
		Penetration	-	25.3	13.9	86	9
Caseinate (0 - 7)	10^{-2}	The effect of surface enlargement	2.7	3.7	-	3	17
		Diffusion	11.1	8.5	0.1	2	34
		Penetration	-	14.7	1.4	8	25
		Penetration	-	19.2	2.8	87	23
Caseinate (0 - 7)	10^{-3}	Diffusion	-	2.0	-	21	19
		The effect of surface enlargement	3.4	4.1	-	13	17
		Diffusion	8.2	9.0	0.5	27	44
		Penetration	-	10.8	4.4	39	20

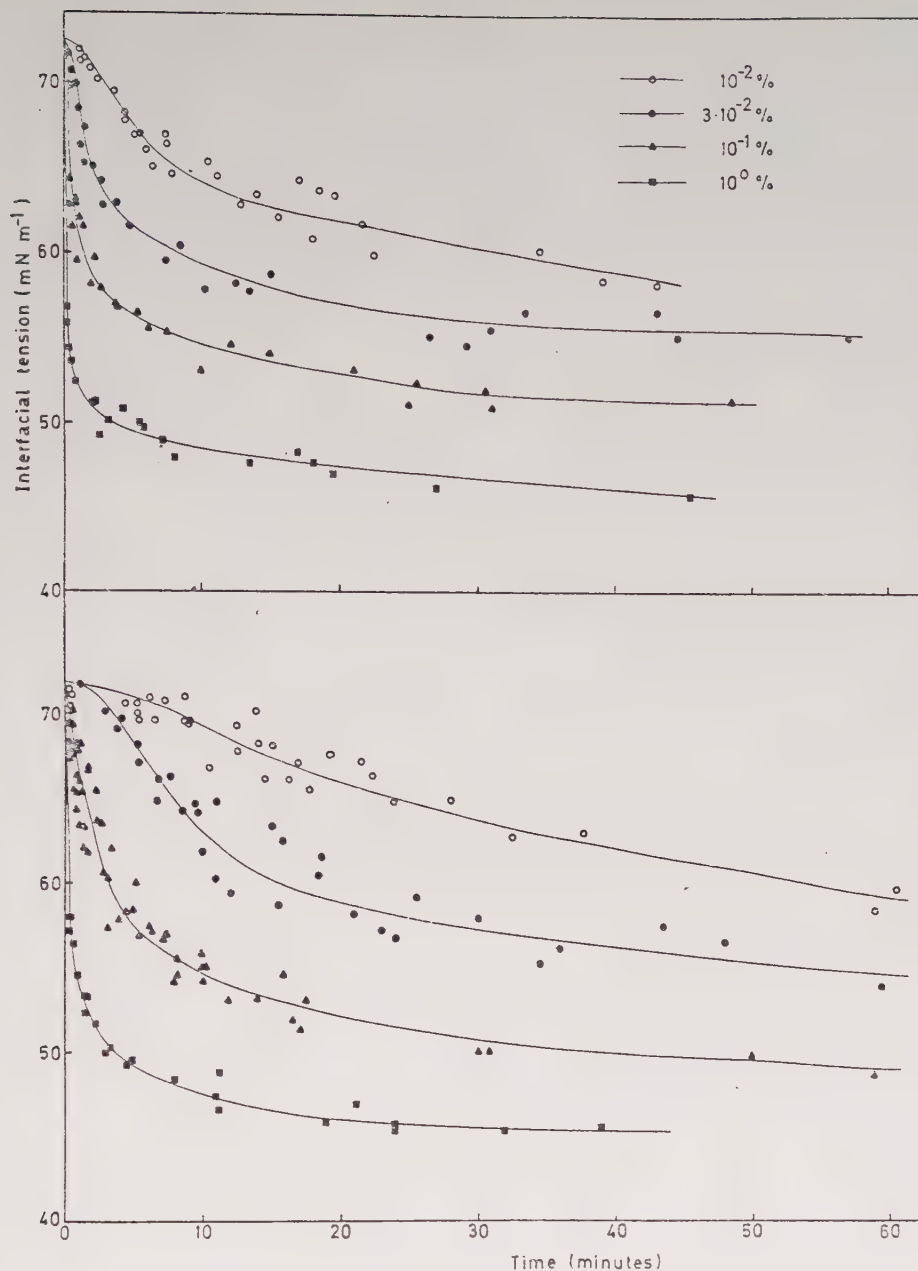


Figure 1 Time-dependence of interfacial tensions at the air-water interface for soy protein at different subphase concentrations. The soy protein is dispersed in 0.2 M NaCl solution in the upper figure and in distilled water in the lower.

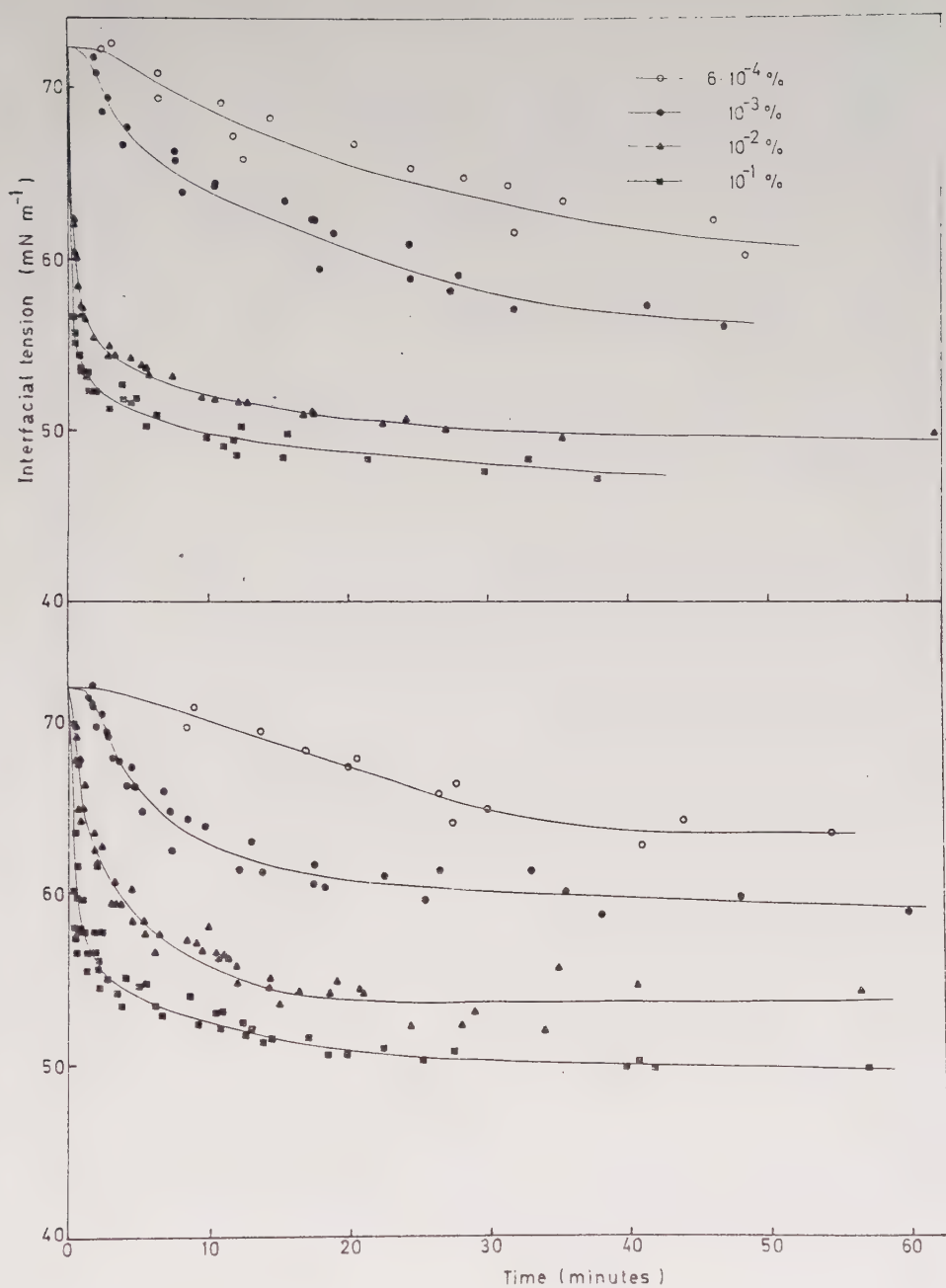


Figure 2 Time-dependence of interfacial tensions at the air-water interface for WPC at different subphase concentrations. The WPC is dispersed in 0.2 M NaCl solution in the upper figure and in distilled water in the lower.

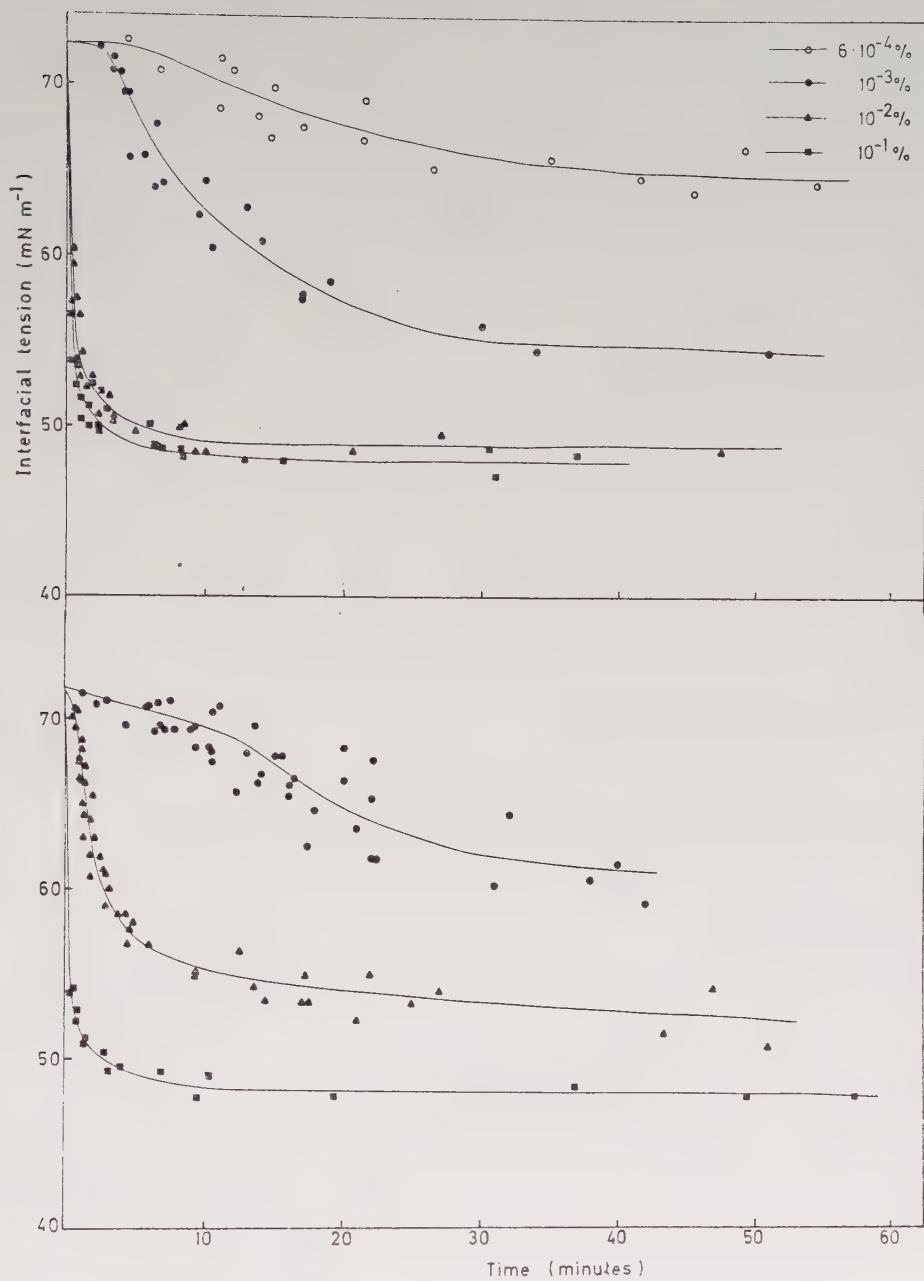


Figure 3 Time-dependence of interfacial tensions at the air-water interface for caseinate at different concentrations. The caseinate is dispersed in 0.2 M NaCl solution in the upper figure and in distilled water in the lower.

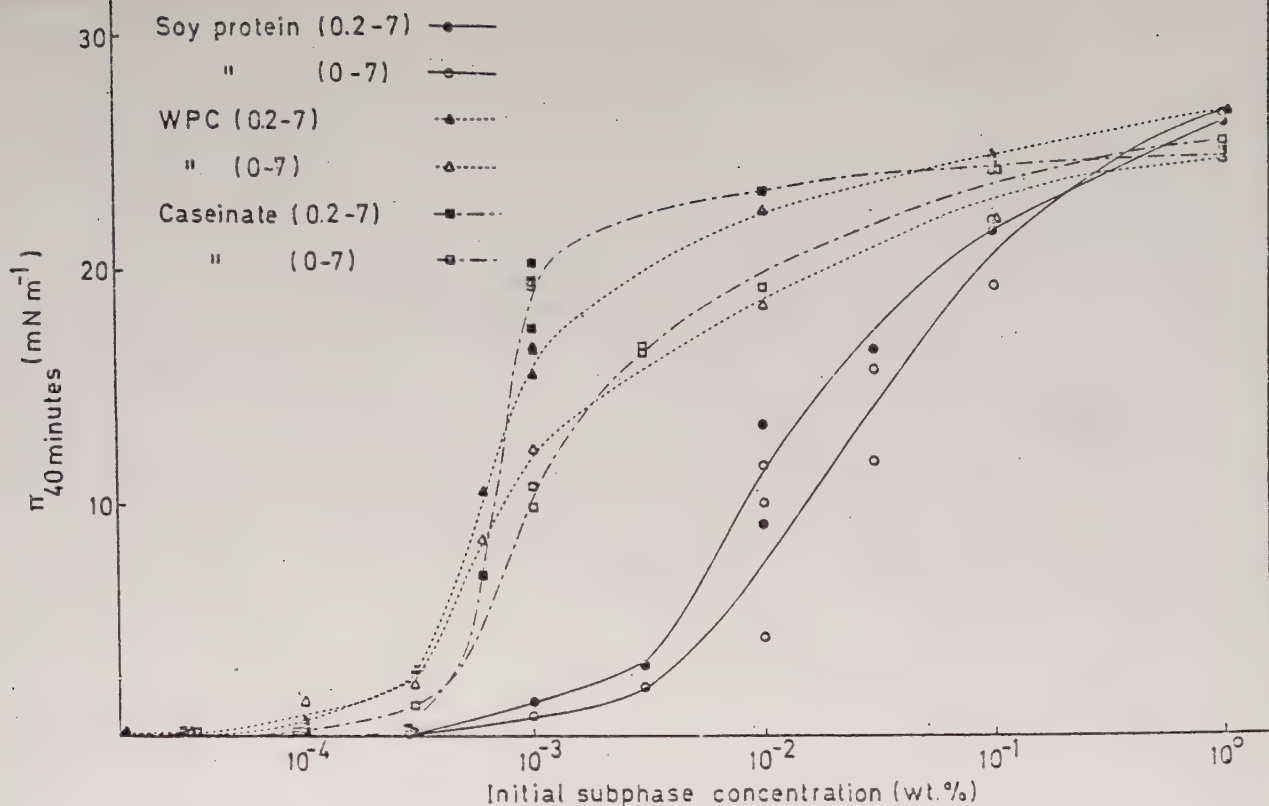


Figure 4 The surface pressure attained after 40 minutes, $\pi_{40 \text{ minutes}}$, as a function of the initial subphase concentration for all the proteins studied at different ionic strengths.

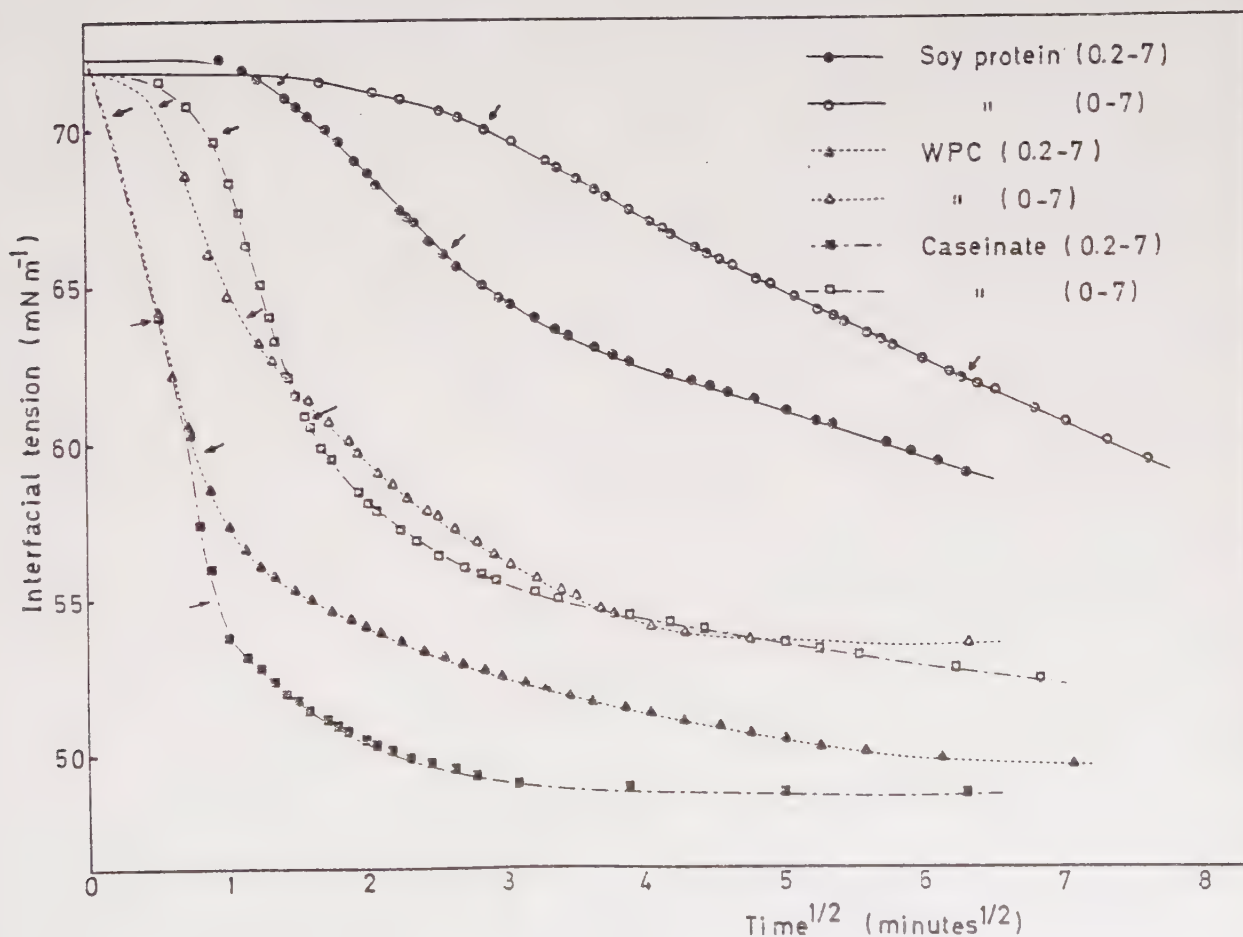


Figure 5 Interfacial tensions at the air-water interface as a function of time^{1/2} for 10⁻² % (w/w) dispersions of soy protein,

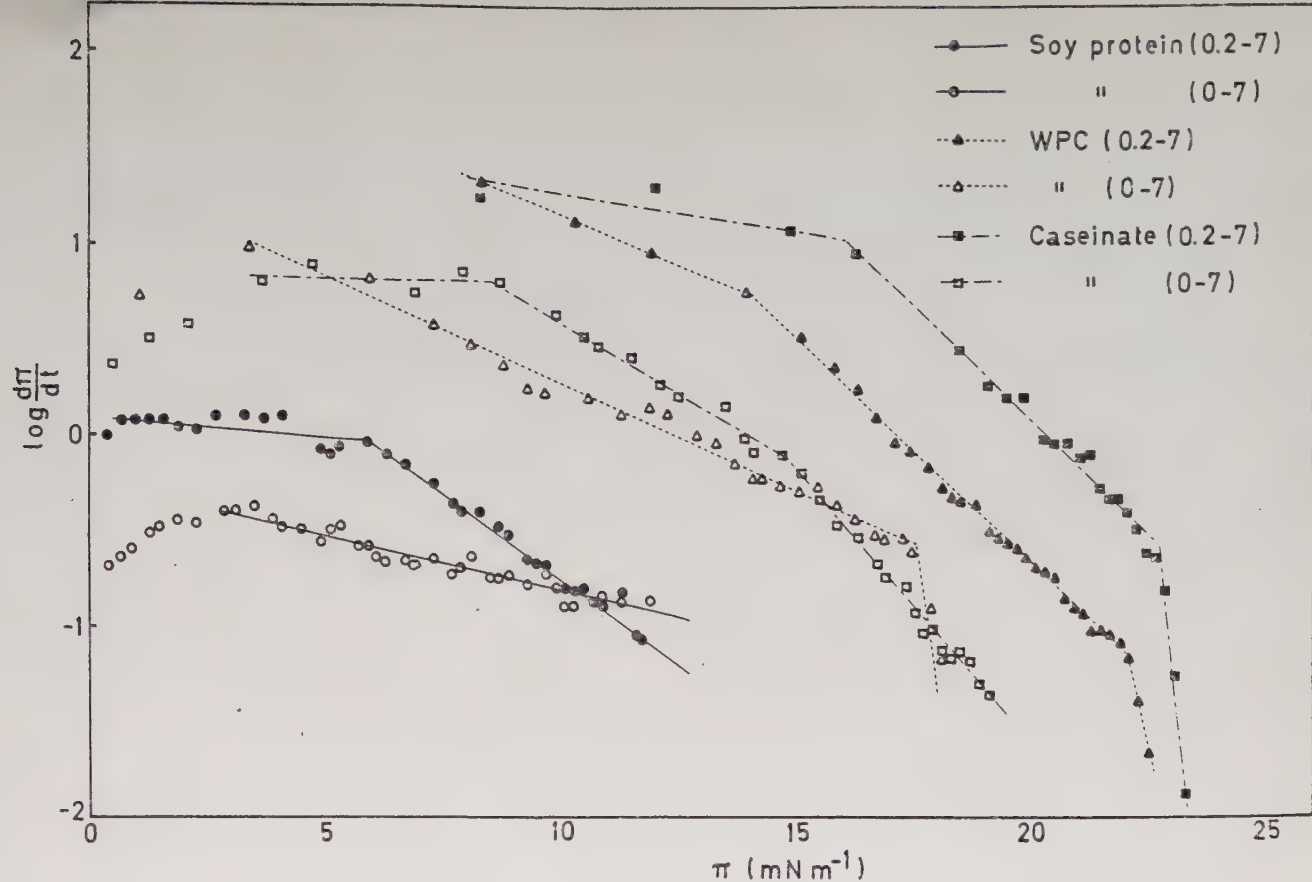


Figure 6 $\log \frac{d\pi}{dt}$ as a function of π for 10^{-2} % (w/w) dispersions of soy protein, WPC and caseinate at different ionic strengths adsorbing at the air-water interface.

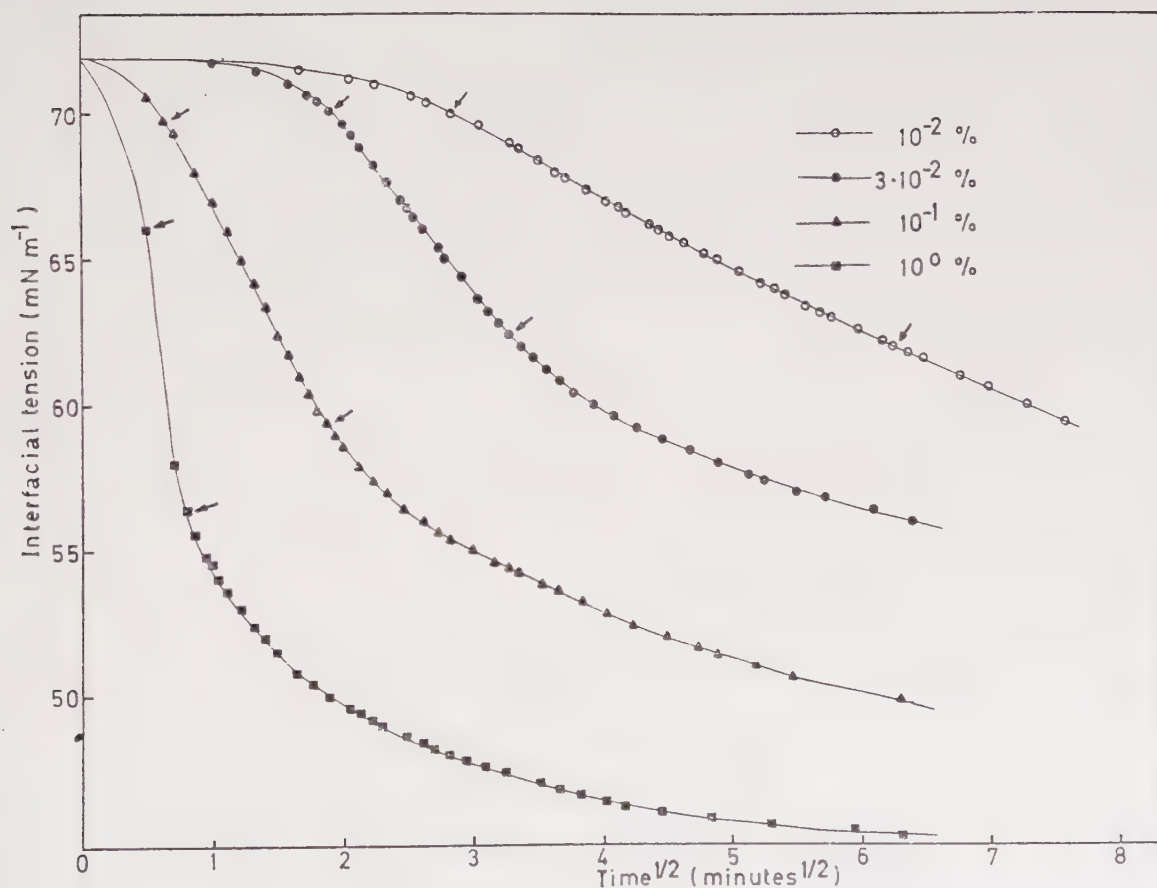


Figure 7 Interfacial tensions at the air-water interface as a function of time^{1/2} for dispersions of soy protein (0 - 7) with

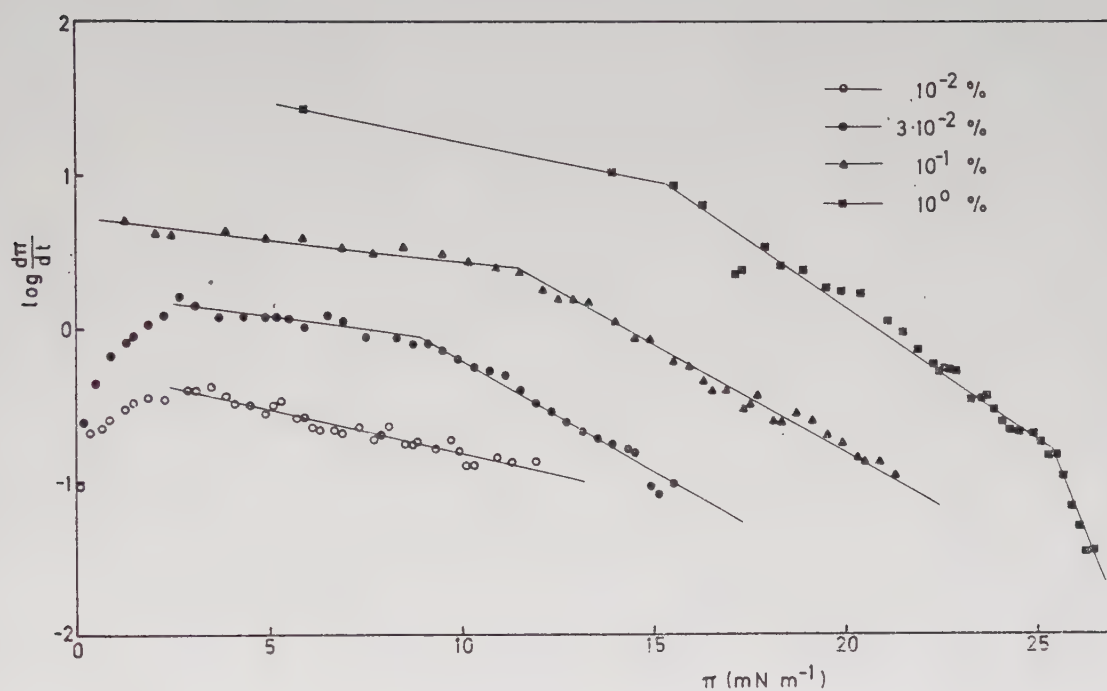


Figure 8 $\log \frac{d\Pi}{dt}$ as a function of Π for dispersions of soy protein (0-7) with a subphase concentration from 1.0 to 0.01% (w/w) adsorbing at the air-water interface.

V

FUNCTIONAL CHARACTERIZATION OF PROTEIN STABILIZED EMULSIONS:
STANDARDIZED EMULSIFYING PROCEDURE

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FUNCTIONAL CHARACTERIZATION OF PROTEIN STABILIZED EMULSIONS:

STANDARDIZED EMULSIFYING PROCEDURE

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ABSTRACT

In order to evaluate the emulsifying properties of proteins it is important to quantify and to more exactly describe the emulsification process. This has been done by utilizing a recirculating emulsification system, where the flow velocity is controlled. The power and energy input during the emulsification process has been measured, thereby providing a tool for the comparison of the emulsifying efficiency of various kinds of emulsifying equipment.

A description of such an emulsification system on a laboratory scale is given for emulsifying with a turbo-mixer, a valve homogenizer and an ultrasonic device.

INTRODUCTION

The importance of the emulsifying procedure in the evaluation of a protein as an emulsifier has previously been shown (Tornberg and Hermansson, 1977). Consequently, the need for better understanding and control of the emulsification process is of vital importance. Firstly, the emulsifying equipment should be standardized so that the flow conditions are comparable. Secondly, it would be desirable to measure power and energy input during the emulsification process, thereby being able to compare the emulsifying efficiency of various kinds of emulsifying equipment.

The purpose of this paper is to describe standardized laboratory apparatus for preparing emulsions, where the flow conditions, power and energy input are controlled and measured.

Mixers of different types, colloid mills, pressure homogenizers and ultrasonic devices are the main tools for emulsification in the food industry. Various mixers find wide application in emulsion technology, and the high speed turbo-mixer is among the most efficient (Brennan, 1970). To produce highly dispersed emulsions both the valve homogenizer and the ultrasonic device can be useful. We have chosen to work with three different types of emulsifying equipment, namely a valve homogenizer, an ultrasonic device and a turbo-mixer.

THE CONTINUOUS FLOW SYSTEM

Insufficient mixing throughout the emulsifying chamber during emulsification can easily cause reproducibility problems. These problems can be reduced in a continuous recirculating system, where the flow velocity is controlled. Continuous emulsification also gives

a way to control and automate the emulsifying procedure; thus, data obtained from this small scale equipment can more easily be scaled up for production requirements. For example, temperature control can be much improved compared to batchwise emulsification. An isothermal emulsification process is obtained by incorporating a heat exchanger unit into the continuous flow system.

Considering these facts, each emulsifying apparatus used is part of an isothermal continuous-flow system with adjustable flow rate and temperature. This system is schematically represented in figure 1, and consists of a gear pump (1) connected to a reversible motor (2) and a revolution counter (3), an emulsifying chamber with in- and outlet (4), a heat exchanger (5) with a thermometer (6) and plastic tubings (7), which complete the recirculating system. The speed of revolution can be varied up to 2 800 rpm with the Multiflex motor (80 W) registered on the Jaquet speed indicator, giving rise to a flow rate of the emulsion up to $1\,050\text{ ml min}^{-1}$. The flow rate of the emulsion through the recirculating system was found to be proportional to the revolution rate of the gear pump.

The heat exchanger is designed as a jacketed cylinder with an inner diameter of the same dimensions as the plastic tubing. The cooling liquid circulates through the jacket and is thermostated by a cryostat (Heto, Denmark). The temperature range employed so far is between 20 and 30°C , and the accuracy of the temperature control is $\pm 2^{\circ}\text{C}$.

The emulsifying part (4) can be varied from emulsification with a turbo-mixer, to sonication and to valve homogenization. The total volume of the circulating system is 52 ml, whereas the volume in the emulsifying chamber can vary from 4.6 ml in the valve homogenizer to 25.2 ml in the turbo-mixer and to 17.5 ml in the ultrasonic device.

The net energy consumption, E , during emulsification in the recirculating system is given by:

$$E = P \cdot N_p \cdot t_p \quad (1)$$

P = net power input (W)

N_p = the average number of passes of an emulsion droplet through the recirculating system

t_p = the average residence time of an emulsion droplet during one passage (s).

The determination of net power consumption varies with the emulsifying apparatus used, and the description of the different procedures is given below. t_p is calculated from the flow rate and the total volume of the recirculating system.

THE EMULSIFYING CHAMBER

Valve homogenizer

Description of the apparatus

After preliminary studies with a valve homogenizer consisting of a spring-loaded conical valve and a hand pump, an improved apparatus was built (figure 2). A spring-loaded (3) entrance valve (2) is situa-

ted just below the feeder funnel (1), to keep the stroke volume, V_s , constant within 4.6 ± 0.2 ml. The entrance valve is a conical, guided valve, to prevent vibration at high pressures (Walstra, 1974) and thereby leaking of emulsion.

The crucial part of the homogenizer is the valve (4) and the valve seat (5). An earlier conical valve was changed to a ball valve of stainless steel, and this greatly improved the pressure maintenance during a stroke. The size of the gap between the valve faces depends upon the flow rate of the emulsion and the loading of the valve, which can be adjusted by compressing a package of disk springs (6) with a screw (7). By replacing the former coil spring with disk springs a better defined spring-load could be obtained.

The pressure applied is registered with an accuracy of ± 0.1 MPa by a strain guage pressure transducer (Bell & Howell) inserted in the valve seat (9). The pressure transducer is connected to a voltmeter, working up to the pressure limit of the apparatus of 20 MPa. The pressure drop can easily be read off on a panel (see figure 3). When pressure changes often and abruptly, as in the case of valve homogenization, it is preferable to use this type of pressure gauge. Cleaning problems are also facilitated compared to other types of gauges.

The plunger (8) is sealed with an O-ring to prevent any leakage of the emulsion. The motion of the plunger is accomplished by a pneumatic system (10), which considerably decreases the size of the entire apparatus compared to a mechanically driven plunger with all its gear equipment. The pressure maintenance during one stroke is also improved by this type of power transmission. All the apparatus can be placed on a table, which makes it easy to operate and to clean. The back and forth motion of the plunger is controlled by two micro-switches (12), which allow the pneumatic system to work continuously.

The continuously working homogenizer is pictured in figure 3. The gear pump is not required in this case, as the homogenizer itself consists of a displacement pump. Provided the stroke volume is constant, the flow rate of the emulsion can be recorded as the number of strokes per unit time; this is achieved by a rod (12) fixed to the plunger, which hits a mechanical counter (13) at every stroke. The stroke rate can be modified by adjusting the air pressure feeding the pneumatic system.

In order to record the pressure profile generated per stroke as a function of the pressure drop and the stroke rate, the pressure transducer was coupled to a storage oscilloscope. Examples of pressure profiles obtained are given in figure 4 at three different pressure drops at a stroke rate of 0.8 stroke/s. Due to the inertia of the system both the form of the pressure profile and the time during which the valve is lifted is dependent on the stroke rate and the pressure drop, as can be judged from figure 4. In figure 5 the time of valve lift during one stroke, t_v , is plotted as a function of the maximum homogenizing pressure, $\Delta p_{\max}$. The figure shows that the

longest times are obtained at the highest pressures and lowest stroke rates.

Measurement of the power input

The net power consumption, P , of the valve homogenizer is equal to the pressure drop, Δp , times the flow rate, Q , of the emulsion, which gives

$$P = \Delta p \cdot Q \quad (\text{J/s, W}) \quad (2)$$

where Δp is the pressure drop in N/m^2 and Q is the flow rate in m^3/s . Q can also be written as

$$Q = V_s \cdot \frac{1}{t_v} \quad (\text{m}^3/\text{s}) \quad (3)$$

where $V_s = (4.6 \pm 0.2) \cdot 10^{-6} \text{ m}^3$ and t_v = time when valve is open during one stroke. Insertion of (3) in (2) gives:

$$P = \Delta p \cdot V_s \cdot \frac{1}{t_v} \quad (\text{W}) \quad (4)$$

As shown by the pressure profiles in figure 4, the pressure drop varies with time during one stroke, which can also be expected for the volume displacement. It is reasonable to assume that the volume displacement, v , is at any moment proportional to Δp , i.e.

$$v = k \cdot \Delta p \quad (5)$$

Integration over the time gives:

$$P = \frac{k}{t_v} \int_0^{t_v} \Delta p(t)^2 dt \quad (6)$$

$$V_s = k \int_0^{t_v} \Delta p(t) dt \quad (7)$$

If a pressure profile is approximated by a parallel trapezium with height $\Delta p_{\max}$, and the shortest parallel side is denoted t_2 and the longest $t_v = t_1 + t_2$, equations (6) and (7) can be written as follows:

$$P = \frac{k}{t_v} \left(\int_0^{t_1} \left(\frac{\Delta p_{\max}}{t_1} \right)^2 \cdot t^2 dt + \int_0^{t_2} \Delta p_{\max}^2 dt \right) \quad (8)$$

$$V_s = k \left(\int_0^{t_1} \frac{\Delta p_{\max}}{t_1} \cdot t dt + \Delta p_{\max} \cdot t_2 \right) \quad (9)$$

After integration the following relationships are obtained:

$$P = \frac{k}{t_v} \left(\frac{\Delta p_{\max}^2 \cdot t_1}{3} + \Delta p_{\max}^2 \cdot t_2 \right) \quad (10)$$

$$V_s = k \left(\frac{\Delta p_{\max} \cdot t_1}{2} + \Delta p_{\max} \cdot t_2 \right) \quad (11)$$

The constant k is obtained from equation (11) and put into equation (10), which gives power, P , equal to:

$$P = \frac{V_s \cdot \Delta p_{\max}}{t_v} \cdot \frac{\left(\frac{t_1}{3} + t_2 \right)}{\left(\frac{t_1}{2} + t_2 \right)} \quad (12)$$

Equation (12) differs from equation (4) only with respect to the last factor $\frac{\frac{t_1}{3} + t_2}{\frac{t_1}{2} + t_2} = f$ in eq. (12). This factor was calculated at

different maximum pressures and strokes rates, and a linear relationship is obtained as a function of $\Delta p_{\max}$ with a correlation coefficient of 0.925:

$$f = - 0.00404 \cdot \Delta p_{\max} + 0.996 \quad (13)$$

where $\Delta p_{\max}$ is expressed in MPa.

The discrepancies in the factor f due to different strokes rates were within the limits of error.

Being a one-piston homogenizer, the transmission of power to the emulsion is made intermittently, hence during liquid flow the net energy input, E , is given by:

$$E = P \cdot N_s \cdot t_v \quad (W) \quad (14)$$

N_s is the number of strokes and t_v is the time of valve lift and can be read off from figure 5.

Estimation of the energy density

Though there are many theories of the mechanism of globule break-up in a valve homogenizer, probably the most important variable determining homogenization efficiency would be the energy density, ϵ_0 , i.e. the power generated per unit volume, depending on pressure and time scale of the process.

Mulder and Walstra (1974) claim that at least in larger machines globules are mainly broken up by small turbulent eddies that cause locally high pressure gradients, which are effective in disrupting

emulsion globules of the same order of size. According to Kiefer (1976) and Treiber (1976) the homogenizing efficiency of a valve homogenizer is mainly attributed to the amount of cavitation occurring. Cavitation is the formation and collapse of small cavities in a liquid, caused locally by pressure drops below the vapor pressure. Collapse of these cavities gives rise to high pressure gradients, and thereby disruption of emulsion globules. Phipps (1974) proposed that it is a viscous shear type of break-up in the elongational flow at the entrance of the valve that causes disintegration into smaller droplets.

According to Walstra (1969), the globule break-up at a certain pressure will primarily depend on the dissipation of the energy in the shortest possible time, which is mainly governed by the valve design. Most of the valve homogenizers commercially available have flat valves, differing in inner and outer radius, giving rise to passage times through the valve seat of about 50 μ s (Walstra, 1974). Most of the energy is probably dissipated within 10 μ s (Walstra, 1974), whereas during the rest of the time in the valve seat coalescence of freshly formed globules occurs (Phipps, 1974). The construction of the homogenizing valve presented here would be likely to have a very short time of the droplets within the valve, as the valve only consists of the circumference of the ball (4) in contact with the valve seat (5). Therefore some rough calculations of the valve lift (l), the time in the valve (τ) and the energy density (ϵ_0) have been performed. The formulas derived for the calculations of l , τ and ϵ_0 are given in appendix. The results are presented in Table 1 for some different pressure drops at a stroke rate of 0.9 stroke/s.

From table 1 it can be concluded that the valve clearance, l , diminishes with increasing homogenizing pressure, which is also the experience of Phipps (1974) and Mulder and Walstra (1974). It is also obvious from the table that the time passages through the valve are comparatively short, all below 10 μ s, which give rise to high energy densities even at relatively low pressures. Walstra (1974) has given an approximate value of ϵ_0 of 500 kW/cm³ for a high-pressure homogenizer, which is surpassed already at a pressure drop of 3.0 MPa. Thus, the homogenizing efficiency can be expected to be very good. As the valve clearances of the apparatus shown here are very small, the mechanism of globule disruption proposed by Phipps can be assumed to occur, which will probably differ from the mechanism taking place in a large-scale homogenizer, where turbulent eddies are supposed to be the main cause of the disruption of globules.

Turbo-mixer

Description of the apparatus

The active part of a turbo-mixer consists of a stationary (stator) and a rotating impeller (rotor), which are both toothed and separated by a small clearance. The liquid circulates through the impellers due to the pumping action exerted by the rotor; it is sheared between both impellers and it impinges upon the slots in them.

A drawing of the apparatus with a thermostating jacket (1) surrounding the emulsifying chamber (2) can be seen in figure 6. In the case of emulsification with the turbo-mixer, the heat exchanger previously described is omitted and the thermostating box (1) is used instead.

The filled arrows show the direction of flow of the cooling liquid, whereas the unfilled arrows indicate the flow of the emulsion. The slots of the stator (4) are somewhat tilted, whereas the slots of the rotor (3) are cut vertically. The shaft (5) to which the rotor is attached runs in bearings, a prelubricated slide bearing (6) and ball bearings (9). To avoid any emulsion squeezed up in the ball bearings, an outlet (7) is placed just below the bearings. The small clearance between the stator and the rotor is adjustable between 0.1 and 1.0 mm, giving rise to differing emulsification processes: both the stator and the rotor are conical, and by moving the shaft (5) upwards, the distance between the rotor and the stator is increased. The shaft is fixed in a position by the guidance screws (10) and the clamping rings (8), and the clearance obtained is read on a scale in vernier graduation (11).

Energy for emulsification comes from the rotor drive motor, a Janke-Kunkel TP 18-10, 170 W, giving a revolution rate up to 20 000 rpm. The rotor speed is monitored by means of a stroboscope, and maintained at the same rate by adjusting a variable transformer.

Measurement of the power input

The measurement of the power generated by the turbo-mixer is not as straightforward as for the valve homogenizer. Usually the power from a mixer is derived by measuring the torque on the shaft and multiply it by the revolution rate. This is not easily done in our case because of the high rotation speed of the shaft. Another way

to approach this problem is to measure the heat dissipated during emulsification. This has been done by incorporating a heater into the continuous recirculating system. The heater consists of a glass tube, on which a resistance wire is wound. Another glass tube with in- and outlets is placed exterior to the first one, thereby forming a small gap between the glass tubes, wherein the rapidly circulating emulsion can be readily warmed up by the resistance wire. The product of the voltage (U) and the amperage (I) through the wire is assumed to determine the amount of power liberated in the heater. A schematic representation of the electronic regulation set up is shown in figure 7. Directly after the heater a thermistor senses the temperature of the emulsion. The cooler is adjusted so that the temperature of the emulsion is kept constant at 25°C . When emulsification takes place, the voltage and the amperage through the heater will diminish in accordance with the amount of heat dissipated in the emulsifying chamber, i.e. the power generated by the turbo-mixer.

Complications that may arise with this sort of apparatus are mainly dependent on the time constant of the system. The time constant can be kept low by a high circulation speed and with a high accuracy of the temperature measurement. Besides, fluctuations in the system, caused for example by entrapped air or by an inhomogeneous emulsion, should be minimized, otherwise the system starts to oscillate. Therefore no measurements could be performed on highly viscous emulsions.

Because of air inclusion and foaming, which sometimes could not be avoided, the accuracy of the measurements was rather low, and the power was determined with an accuracy of ± 1 W. It was found that

between 15 000 and 20 000 rpm the power transmitted to the emulsion by the turbo-mixer varied from 7 to 12 W.

Estimation of the energy density

Mulder and Walstra (1974) claim that both turbulence and cavitation are produced, and the effective energy density is estimated to be around 200 W/cm^3 (Walstra, 1975).

Ultrasonic device

Description of the apparatus

A Branson Sonifier Cell Disruptor model B-12 (Branson Sonic Power Co.) was used. To fit it into the recirculating system a continuous-flow attachment of stainless steel from the same firm was used. The attachment is screwed onto the horn, and the distance from the tip of the horn to the outlet can be varied by unscrewing, with each complete revolution corresponding to a distance of 0.8 mm. By this procedure the size of the space where most of the emulsification takes place can be changed, and thereby the emulsification performance modified.

Measurement of the power input

The two most commonly used methods of measuring ultrasonic power are calorimetry and the radiation force method (Zieniuk and Chivers, 1976). Although the calorimetric method has a better theoretical basis, the radiation force method is more rapid and convenient to use. For this reason we decided to mainly use the radiation force method, although some measurements of the power were performed with the previously described calorimeter.

In the radiation force method one measures the radiation pressure force, F , exerted on a wall placed at a right angle in the path of an ultrasonic beam with a power, P . Eqn. (15) is obtained,

$$F = \frac{P}{c} \quad (N) \quad (15)$$

where c is the velocity of sound in the medium in which the beam travels. Wemlén (1968) has calculated that if oil is used as the propagating medium with c equal to 1377 m/s the radiation force expressed in weight is 74 μg for an ultrasonic power of 1 mW. As the velocity of sound in water is 1 500 m/s, a radiation force of approximately 72 μg corresponding to a power of 1 mW, is obtained for an o/w-emulsion of 40% oil by weight.

Zieniuk and Chivers (1976) have discussed probable errors that can be introduced in the radiation force method, and they came to the conclusion that at present it would appear to be both convenient and correct for practical purposes to assume that equation (15) is valid.

The experimental set up consists of an open vessel containing emulsion, an electronic balance (Sartorius 3700) with an analog output, a recorder, and the ultrasonic device. The plastic vessel has a bottom diameter of 65 mm and the walls are covered with a 15 mm thick plastic foam. Assuming that the bottom of the vessel behaves like a perfect reflector of the ultrasound beam, the plastic foam on the walls of the vessel should behave like a perfect absorbant. That this could be justified within the limits of error was found by preliminary

experiments, in which the model of the vessel given above was independent of the thickness of the plastic foam and of the distance from the bottom to the ultrasonic horn dipped into the emulsion.

The analog output of the balance was coupled to the recorder, and the power generated by the ultrasonic horn could be detected with an accuracy of ± 1.5 W. During the experiments the emulsion was cooled now and then in order to minimize the influence of temperature on the measurements.

In figure 8 the power measured is plotted as a function of the apparatus parameter, control setting. Power has been measured for emulsions of soybean oil (40 wt. %) in water, where soy protein isolate, whey protein concentrate (WPC) and sodium caseinate have been used as stabilizers. The number in brackets (0 - 7) and (0.2 - 7) denote when the protein is dispersed in water or in 0.2 M sodium chloride solution at pH 7. Minor variation due to the protein used as stabilizer can be viewed from figure 8, but if this is taken into account the net power consumption can be determined with an accuracy of ± 3 W. For comparison, measurements made with the calorimeter are also given in figure 8 for emulsions stabilized by caseinate (0 - 7) and caseinate (0.2 - 7). As can be seen from the figure, the values obtained from the calorimeter are higher at low control settings, whereas they more or less coincide for increasing values of the control setting. The discrepancies between the values obtained by the two different methods are most probably due to insufficient accuracy of the thermistor in the calorimeter.

Estimation of the energy density

An ultrasonic horn generates ultrasonic waves, which produce cavitation, i.e. the formation and collapse of small cavities in the liquids. When these cavities collapse high pressure gradients arise, which cause globule disruption. The energy density produced can be as much as approximately 1 MW/cm^3 (Walstra, 1974).

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REFERENCES

- Brennan, J.G. 1970. Emulsions in Food Technology. Process Biochem. July, 33.
- Kiefer, P. 1976. Experimentelle Bestimmung des Zerkleinerungsmechanismus Turbulenz bei der Hochdruckhomogenization von O/W-Emulsionen. Z. Lebensmitteltechnologie und -Verfahrenstechnik 27: 12.
- Mulder, H. and Walstra, P. 1974. The milk fat globule. Emulsion science, as applied to milk products and comparable foods. Pudoc, Wageningen and Commonwealth Agric. Bureaux, Farnham Royal.
- Phipps, L.W. 1974. Cavitation and separated flow in a simple homogenizing valve and their influence on the break-up of fat globules in milk. J. Dairy Res. 41: 1.
- Tornberg, E. and Hermansson, A.-M. 1977. Functional characterization of protein stabilized emulsions: Effect of processing. J. Food Sci. 42: 468.
- Treiber, A. 1976. Einfluss der Kavitation als Zerkleinerungsmechanismus bei der Hochdruckhomogenization von O/W-Emulsionen. Z. Lebensmitteltechnologie und -Verfahrenstechnik 27: 11.
- Walstra, P. 1969. Preliminary note on the mechanism of homogenization. Neth. Milk Dairy J. 23: 290.
- Walstra, P. 1974. Personal communication.
- Walstra, P. 1974. Influence of rheological properties of both phases on droplet size of o/w emulsions obtained by homogenization and similar processes. Dechema Monographie 77: 87.
- Walstra, P. 1975. Effect of homogenization on the fat globule size distribution in milk. Neth. Milk Dairy J. 29: 279.

Wemlén, A. 1968. A milliwatt ultrasonic servo-controlled balance.

Med. & Biol. Engng. 6: 159.

Zieniuk, J. and Chivers, R.C. 1976. Measurement of ultrasonic exposure with radiation force and thermal methods. Ultrasonics, July.

APPENDIX

Calculation of l , t and ϵ_0 in the valve homogenizer

By assuming that pressure tends towards zero somewhere inside the valve, the average maximum velocity, v_m , can roughly be estimated from Bernoulli's law:

$$v_m \approx \left(\frac{2 \Delta p_{\max}}{\rho} \right)^{1/2} \quad (1)$$

The average maximum velocity, v_m , can also be calculated from the flow rate, $Q = \frac{V_s}{t_v}$, divided by A_t , the minimum area the emulsion must pass when traversing the valve.

$$A_t \approx 2 \pi R l \quad (2)$$

In this equation, R is the radius of the circumference of the ball valve which is in contact with the valve seat and equals to 0.5 cm.

l can now be extracted from the following relationship:

$$v_m = \frac{V_s}{t_v \cdot 2 \pi R l} = \left(\frac{2 \Delta p_{\max}}{\rho} \right)^{1/2} \quad (3)$$

The volume inside the valve, V_i , is given by:

$$V_i = 2 \pi \cdot R \cdot a \cdot l \quad (4)$$

where $R = 0.5$ cm and a is the length of the valve seat and can be considered not to exceed 0.05 cm.

The time in the valve seat, τ , can then be calculated according to:

$$\tau = \frac{V_i}{Q} = \frac{2 \pi R \cdot a \cdot l \cdot t_v}{V_s} \quad (5)$$

The energy density, ε_0 , can be estimated from the following equation:

$$\varepsilon_0 = \frac{\Delta p_{\max}}{\tau} \quad (6)$$

Table 1. Calculated values of the valve lift, time in the valve seat and energy density at different maximum pressure drops for a stroke rate of 0.9 stroke/s.

Maximum pressure drop $\Delta p_{\max}$ (MPa)	Valve lift l (μm)	Time in the valve τ (μs)	Energy density ϵ_0 (kW/cm^3)
1.0	7.1	9.0	110
2.0	4.5	6.3	315
3.0	3.4	5.2	580
5.0	2.3	4.0	1250
7.5	1.7	3.3	2300
10.1	1.4	2.8	3570

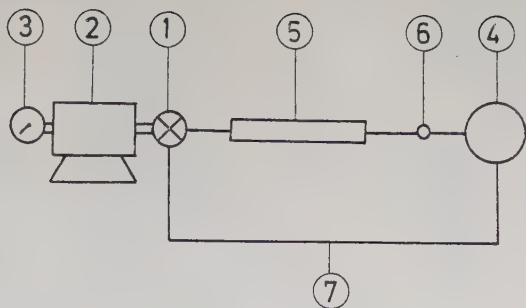


Figure 1. A schematic representation of the isothermal recirculating, emulsification system.

- 1 GEAR PUMP
- 2 REVERSIBLE MOTOR
- 3 REVOLUTION COUNTER
- 4 EMULSIFYING CHAMBER
- 5 HEAT EXCHANGER
- 6 THERMOMETER
- 7 PLASTIC TUBINGS

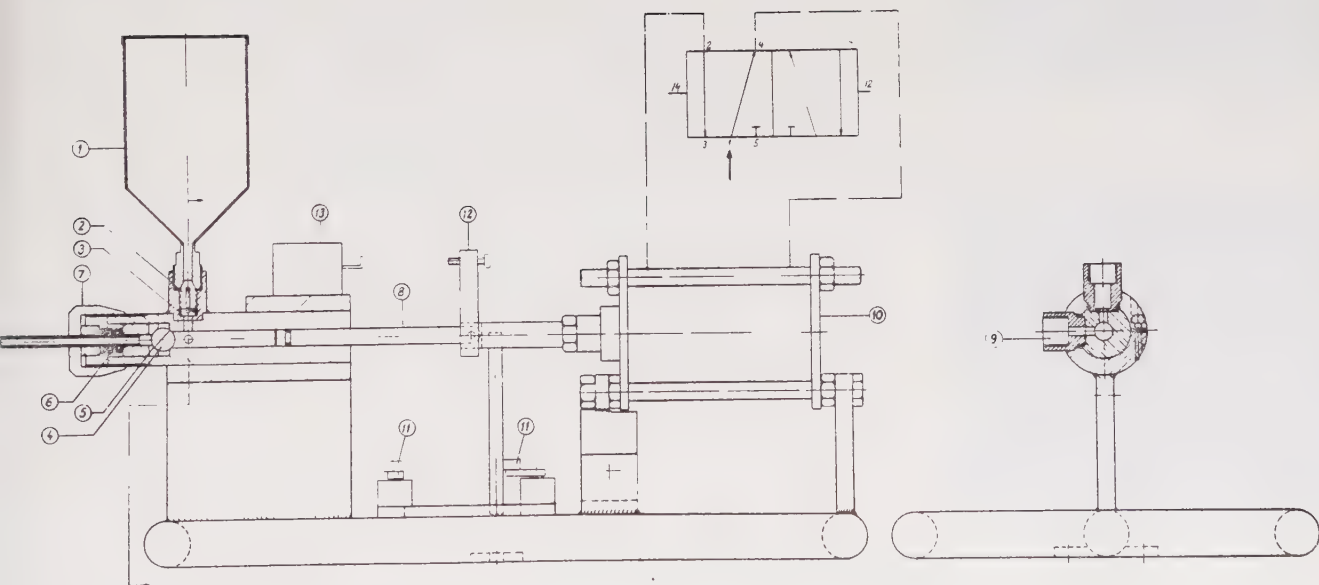


Figure 2. A construction drawing of the valve homogenizer.

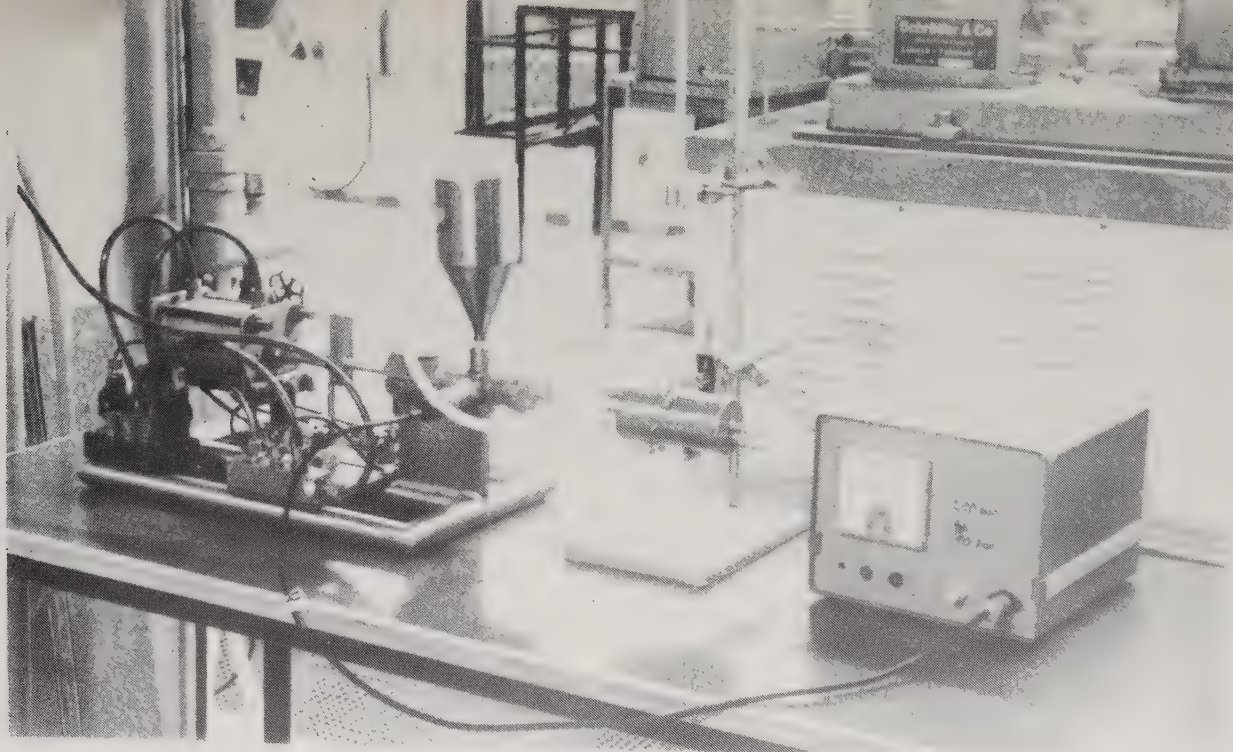
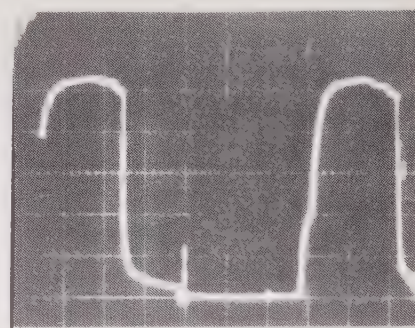


Figure 3. The apparatus set-up of the recirculating emulsification system using the valve homogenizer.

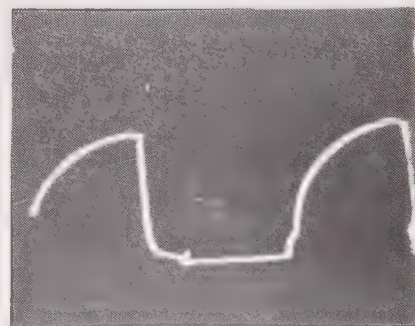
Figure 4. Examples of pressure profiles obtained per stroke with the valve homogenizer at a stroke rate of 0.8 stroke/s.

- A : Pressure drop = 2.5 MPa;
Vertical = 1 mV/division;
Horizontal = 0.2 s/division
- B : Pressure drop = 7.5 MPa;
Vertical = 5 mV/division;
Horizontal = 0.2 s/division
- C : Pressure drop = 15 MPa;
Vertical = 5 mV/division;
Horizontal = 0.2 s/division

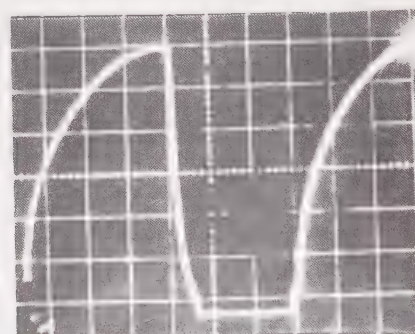
A



B



C



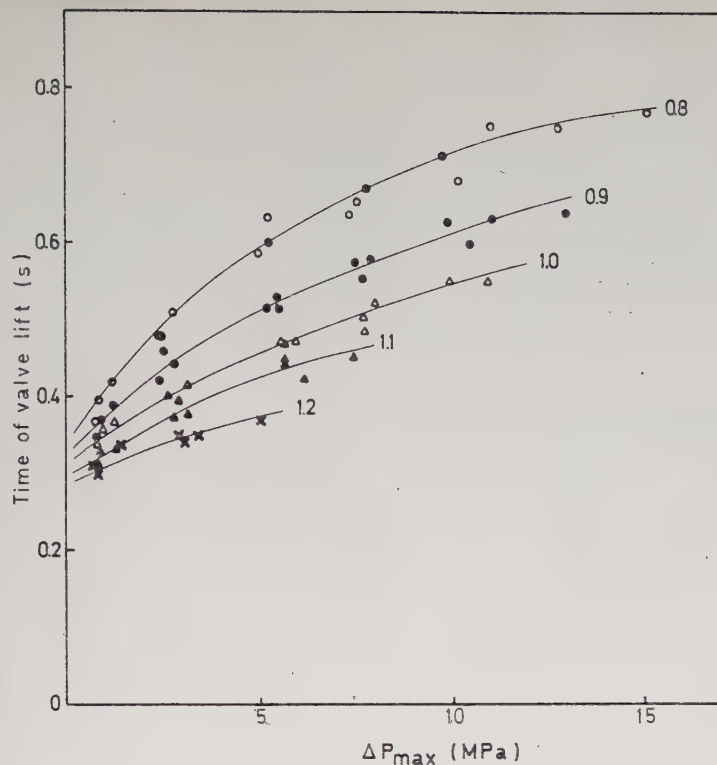


Figure 5. The time of valve lift, t_v , in the valve homogenizer as a function of the maximum homogenizing pressure, $\Delta p_{\max}$.

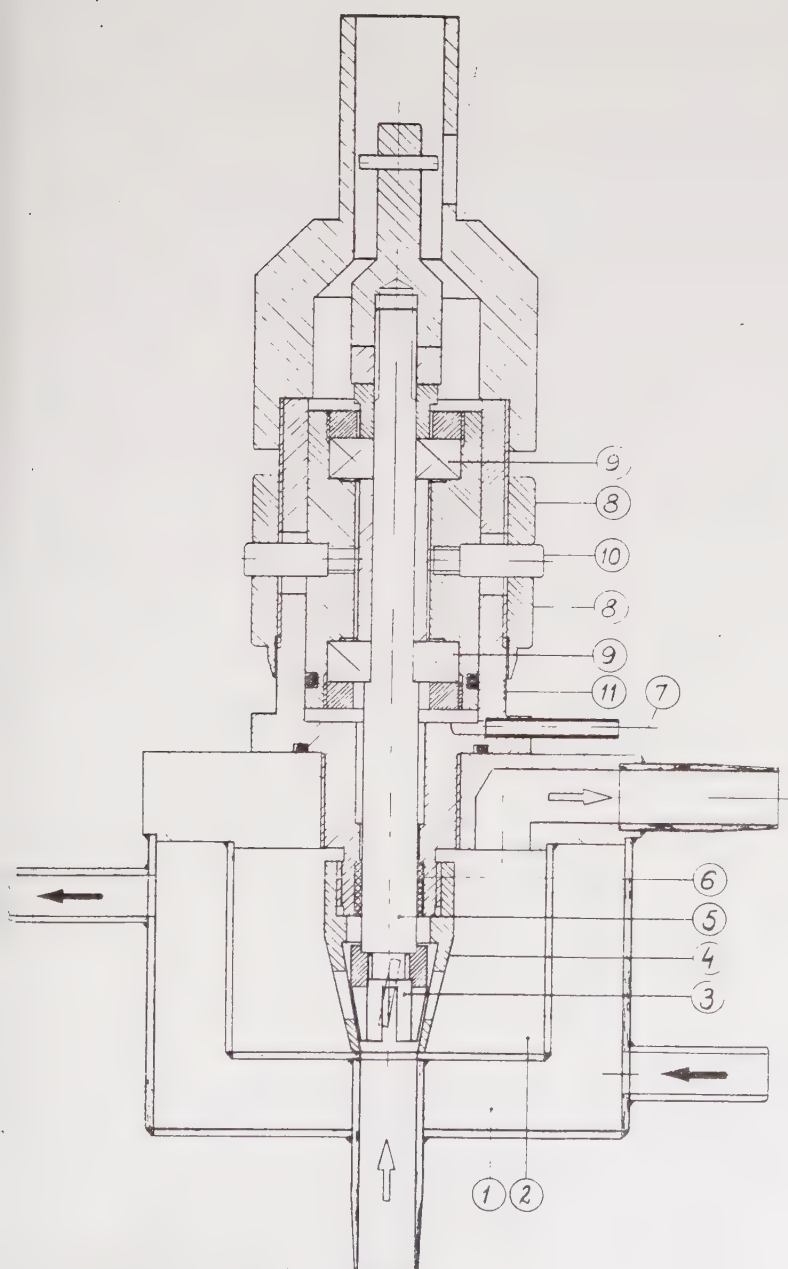


Figure 6. A construction drawing of the turbo-mixer.

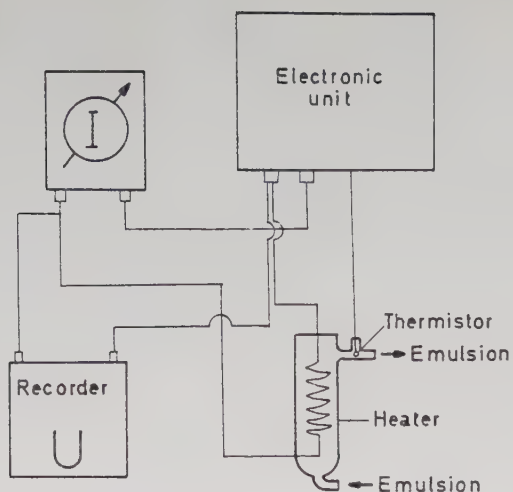


Figure 7. A schematic representation of the electronic unit within the set up for calorimetry.

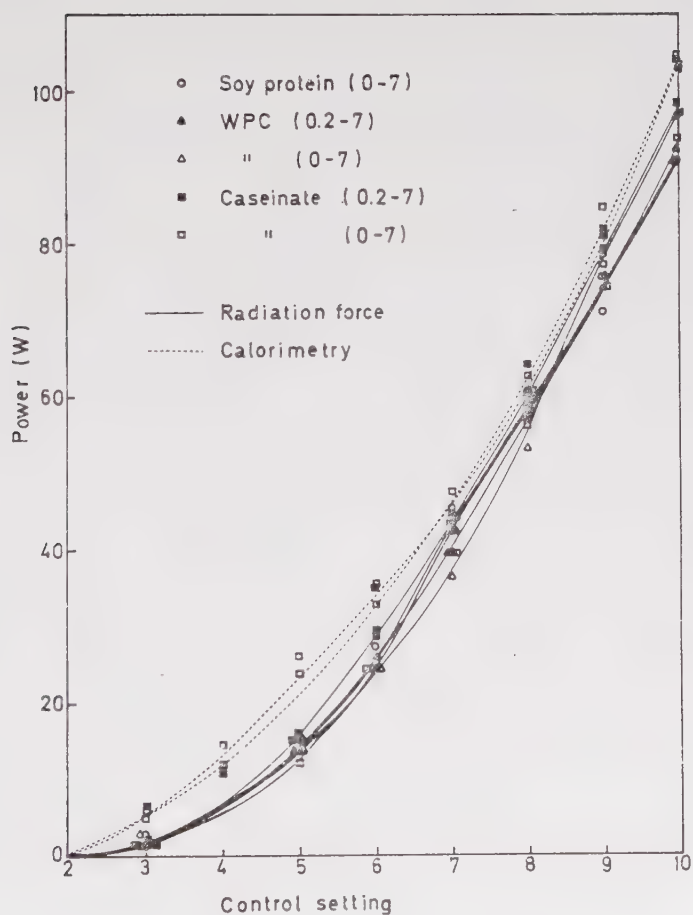


Figure 8. The ultrasonic power as a function of control setting for different protein stabilized emulsions measured with both the calorimetry and the radiation force method.

FUNCTIONAL CHARACTERIZATION OF PROTEIN STABILIZED EMULSIONS:

CREAMING STABILITY

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CREAMING STABILITY

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ABSTRACT

Protein stabilized emulsions have been prepared in a recirculating emulsification system, where flow velocity, power and energy input have been controlled and measured. Three different types of emulsifying equipment have been used, namely a turbo-mixer, an ultrasonic device and a valve homogenizer. The protein systems studied were a soybean protein isolate, a whey protein concentrate (WPC) and a sodium caseinate, and the emulsions obtained were characterized in terms of creaming stability.

It was found that although the power and energy consumption during emulsification were the same, the creaming stabilities differed as a function of the emulsifying apparatus used. Increased power and energy input contributed in general to an improved creaming stability up to a certain limit, whereupon it leveled off. The emulsifying efficiency of the turbo-mixer is poorest in terms of creaming stability of the emulsions formed, whereas the ultrasonic device most generally is the best choice of equipment at lower power input. At an increase of power consumption the valve homogenizer is an equally good alternative, or even better.

INTRODUCTION

The emulsifying properties of proteins have been subject to considerable study, but no clear picture has so far emerged from the findings. The fact that the mode of formation of the emulsions greatly influences their properties (Tornberg and Hermansson, 1977) may be one reason that so many contradictory results are to be found in the literature, since very little attention has been paid to this variable. To be able to deal with the parameters of emulsification, the process must be standardized and quantified. This has been done by using a recirculating system, where the flow velocity, power and energy input are controlled and measured. This emulsification system is described elsewhere (Tornberg and Lundh, in press).

In this paper, the creaming stability of protein-stabilized emulsions has been investigated as a function of the energy input, the power input and the number of passes through a recirculating system during emulsification. Three different pieces of emulsifying equipment, a turbo-mixer (T-M), a valve homogenizer (V-H) and an ultrasonic device (U-S), have been used. The emulsions have been stabilized by three protein products, namely a soy protein isolate, a sodium caseinate and a whey protein concentrate (WPC).

MATERIAL

Soy protein isolate

A soy protein isolate produced under mild conditions was kindly provided by Central Soya. It was prepared from defatted soy bean flakes by extraction with deionized water, precipitation at pH 4.6, washing the curd with deionized water and neutralizing to pH 7.0.

Analysis: protein (N x 6.25) 96.0% (dry weight); solubility determined according to Hermansson (1973) in distilled water and in 0.2 M sodium chloride solution at pH 7, denoted as (0 - 7) and (0.2 - 7), is 91.7% and 76.1%, respectively.

Caseinate

Spray blend caseinate (DMV, Holland), a commercially available sodium caseinate, was used. Analysis: protein (N x 6.43) 92.6% (dry weight); solubility determined as above in distilled water and in 0.2 M sodium chloride solution at pH 7, denoted as (0 - 7) and (0.2 - 7), is 97.4% and 95.4%, respectively.

Whey protein concentrate (WPC)

The whey protein concentrate was obtained by ultrafiltration and spray drying of cheese whey. UF was carried out at 5°C in a pilot plant unit from De Danske Sukkerfabriker (DDS) with a membrane area of 2.2 m² and with a membrane type designated as 600 DDS. Analysis: protein (N x 6.55) 68.4% (dry weight); solubility determined as above in distilled water and in 0.2 M sodium chloride at pH 7, denoted as (0 - 7) and (0.2 - 7), is 100% and 98.2%, respectively.

Soybean oil

A commercially available soybean oil (AB Karlshamns Oljefabriker, Karlshamn, Sweden) was used. Analysis: fatty acid composition 18:2 53.3%, 18:1 23.0%, 16:0 10.8%.

METHODS

Preparation of samples

Protein dispersions (2.5% (w/w), based on the protein content) in distilled water or sodium chloride solution were made with the

Sorvall omni-mixer. The pH was adjusted with 0.2 M NaOH or 0.2 M HCl. Soybean oil was added directly to the protein dispersion to attain 40% oil by weight.

Emulsion formation

A quantity of 50 grams of emulsion was emulsified in a recirculating system as previously described (Tornberg and Lundh, in press). The emulsifying part was varied from a turbo-mixer, to a valve homogenizer and to an ultrasonic device. A thorough description of each the emulsifying units and the measurement of the power and energy input has been given by Tornberg and Lundh (in press). The flow rate of the emulsion through the recirculating system was held constant at $250 \pm 25 \text{ ml min}^{-1}$, giving an average residence time of an emulsion droplet during one passage, t_p , of $12.5 \pm 0.4 \text{ s}$, and an average stroke rate of 0.9 stroke/s in the valve homogenizer. The distance from the tip of the ultrasonic horn to the outlet of the continuous flow attachment was set to 0.8 mm.

Cooling was performed during all the emulsification procedures to keep the temperature of the emulsion near 25°C during processing.

Emulsion characterization

The extent of creaming is called the stability rating (SR). It was determined on the basis of the percentage change of fat in the aqueous lower phase after creaming for 24 hours of the emulsions formed. The description of the method and the calculations are given elsewhere (Tornberg and Hermansson, 1977). The accuracy of the method is $\pm 1.5\%$ SR.

RESULTS

Effect of energy input

In figure 1 the creaming stabilities of differently emulsified WPC-(0.2 - 7)-stabilized emulsions are plotted as a function of the net energy input. The power consumption has been held constant at levels of 10 and 40 W, and the number of passes has been varied during emulsification. Firstly, we can establish that although the same amount of energy has been transferred to the emulsion during emulsification, the creaming properties differ considerably as a function of the emulsifying equipment and the power generated. Secondly, an increase in energy input gives rise to better emulsions. At a power transmission of about 10 W the sonicated emulsions are superior in creaming stability to the valve homogenized ones at all energy levels, and these are in turn better than the emulsions made in the turbo-mixer. Interesting to note is the difference in creaming behaviour of the emulsions on sonication or valve homogenization as the power consumption is increased to 40 W. Now the valve homogenizer is a better alternative, and the sonicated emulsions at this level of power consumption are even worse in creaming stability than the emulsion made at 10 W for the same amount of energy input. Similar creaming behaviour as a function of net energy consumption, at a constant power supply, has been observed for WPC-(0 - 7)-, caseinate-(0.2 - 7)- and soy protein-(0.2 - 7)-stabilized emulsions.

In figure 2 the creaming stabilities of caseinate-(0.2 - 7)-stabilized emulsions are plotted as a function of net energy consumption. In this case, the number of passes has been held constant and the power

consumption has been varied. Here again the ultrasonic equipment and the valve homogenizer behave very differently, when the number of passes is increased from 10 to 30. For the valve homogenizer it is more energetically favourable to use a lower number of passes, whereas there is nothing to gain in energy consumption by lowering the number of passes if the emulsions are sonicated. Another interesting feature visible in figure 2 is the difference in form of the curves obtained by sonicating and valve homogenizing the emulsions; the former increases only slowly with higher energy input, whereas the latter changes more abruptly in a certain range of energy input. Similar curves to those in figure 2, when using the same variables, have also been obtained for WPC-(0.2 - 7)- and soy protein-(0 - 7)-stabilized emulsions.

Effect of power input

In figure 3 the stability ratings of soy protein-(0.2 - 7)-stabilized emulsions are plotted as a function of the net power consumption for sonicated and valve homogenized emulsions at 10 and 30 passes, respectively. The wide scatter of results derived is probably due to differences in viscosity, as the emulsions got very viscous when formed, as visually judged. In the ultrasonic device, the viscosity increase is so high that the emulsions could not circulate at a power input beyond 20 W. It can also be deduced from figure 3 that the valve-homogenized products at 30 passages tend to be over-processed in terms of creaming stability.

As expected for the two types of emulsifying equipment, an increase in power input improved the creaming stability of the emulsions up to a certain level, whereupon it levels off. But the steep rise in stability rating occurs at a lower power input for the sonicated

emulsions than for the valve-homogenized ones. In figure 4 the emulsification efficiency of the valve homogenizer and the ultrasonic device at 30 passages have been compared for soy protein-(0 - 7)-, WPC-(0 - 7)- and caseinate-(0.2 - 7)-stabilized emulsions. It is obvious that the ultrasonic device is superior to the valve homogenizer in the low power region below 20 W. Although all emulsions shown in figure 4 reach the plateau value of $\approx 90\%$ SR, the rate of increase of the creaming stability as a function of power input differs between emulsions stabilized with different proteins. As judged from figure 4, WPC-(0 - 7)-stabilized emulsions reach the plateau value at the lowest power input, closely followed by soy protein-(0 - 7)-, whereas caseinate-(0.2 - 7)-stabilized emulsions have the lowest creaming stabilities up to 40 W.

One protein that stabilizes emulsions with completely different creaming behaviour than the other proteins used, is caseinate-(0 - 7). This becomes evident on comparing figures 4 and 5, where in the latter figure the stability rating of caseinate-(0 - 7)-stabilized emulsions is given as a function of power consumption at a constant number of passages of 10 and 30. It is clear from figure 5 that the improvement in creaming stability on increasing the power input is comparatively slow for caseinate-(0 - 7). A value as high as about 70 W is needed to reach the plateau value for 30 passages, whereas the other proteins only need 10 - 40 W to attain the same level of creaming stability. The difference in emulsifying behaviour of the ultrasonic device and the valve homogenizer is also very clear from figure 5. Sonication gives emulsions with a creaming stability that almost linearly increases with power consumption, whereas valve homogenization gives rise to emulsions in which the stability rating increases more abruptly with power input.

All the protein stabilized emulsions investigated are compared for valve homogenization at 10 passages in figure 6, where stability rating is given as a function of power consumption. It can be concluded from the figure that the addition of salt most favourable increases the creaming stability of caseinate-stabilized emulsions, whereas the opposite is found for the other two proteins. The same order in creaming stability as found in figure 4 can also roughly be estimated from figure 6, i.e. WPC gives emulsions of highest SR followed by soy protein isolate and thereafter sodium caseinate.

Effect of number of passes

In figure 7 the stability ratings of WPC-(0 - 7)-stabilized emulsions are plotted as a function of number of passes at constant levels of about 10 and 40 W power input. Some features, typical for all the protein stabilized emulsions, can be seen in this figure: At the lower power input the ultrasonic equipment gives emulsions in which the rate of growth of creaming stability is very high compared to the valve-homogenized emulsions; the emulsions obtained at 40 W are always superior in creaming stability to those obtained at 10 W for the same number of passes. Also, increasing the number of passes improves the creaming stability of the emulsions formed. A property of the valve-homogenized WPC-(0 - 7)-stabilized emulsions at 40 W not observed to the same extent for the other protein stabilized emulsions is the extreme sensitivity of the creaming properties to an increase in the number of passes in the range 1 - 3.

A comparison of the emulsifying efficiency of both the emulsifying machines can be made at a power input of 40 W in figure 8, where stability ratings obtained for soy protein-(0 - 7)-, WPC-(0.2 - 7)-

and caseinate-(0 - 7)-stabilized emulsions are plotted as a function of number of passes. For both the caseinate-(0 - 7) and the soy protein-(0 - 7), the ultrasonic device is more efficient, whereas the reverse is found for WPC-(0.2 - 7). Apparently, the valve homogenizer is a good choice of emulsifying equipment for the preparation of creaming stable WPC-stabilized emulsions. This is also verified by figure 9, where all the proteins can be compared as emulsifiers on valve homogenization at 10 W. The axes are the same as in figures 7 and 8. It is obvious from the figure that especially the WPC (0 - 7) stabilized emulsions more readily get a higher creaming stability as the number of passes is increased. The same roughly estimated order as found in figures 4 and 6 also becomes apparent in figures 8 and 9.

DISCUSSION

In this investigation the creaming behaviour of protein-stabilized emulsions formed in different ways under controlled conditions has been studied. An evaluation of the factors affecting the creaming behaviour of the emulsions can be made by comparison of studies made on milk systems. Walstra and Oortwijn (1975) have found that viscosity and globule size are the most important variables in determining the creaming behaviour of unclustered milk, and that increased polydispersity generally decreases the creaming rate.

To be certain that the protein itself was not severely affected by the high energy densities introduced during the emulsification process, some preliminary experiments were performed. The protein dispersions used in this investigation were subjected to the same emulsification procedures as the emulsions formed, and the turbidi-

ty and the solubility of the dispersions were measured before and after treatment. No profound irreversible changes in the properties of the protein dispersions were observed. Mulder and Walstra (1974) report that at high pressures and with large micelles disruption of casein micelles can take place in the homogenizer. But the preparation of sodium caseinate destroys the original properties of the casein micelle and possibly also the structure of the submicelles (Schmidt and Buchheim, 1975). Consequently, disruption of the sodium caseinate in the dispersion during emulsification can be considered negligible. It is assumed that the properties of the emulsion formed is mainly determined by the dynamic flow conditions during emulsification and by the way in which the proteins act at the interface under these circumstances.

The type of emulsifying equipment and power input determine the energy density, ϵ_0 , generated, i.e. power input per unit volume, and these values have been estimated in a preceding paper (Tornberg and Lundh, 1977) for the three emulsifying machines used.

The emulsifying efficiency of the turbo-mixer is low compared to that of the other two pieces of emulsifying equipment (see figure 1). This could be expected as both the estimated energy density is low ($\approx 200 \text{ W/cm}^3$) and a very wide spread in globule size is often obtained (Mulder and Walstra, 1974). The differences in creaming behaviour of emulsions produced by the valve homogenizer and the ultrasonic device is mainly due to the latter giving emulsions with very small particles but a wide spread in globule size, compared to the former (Mulder and Walstra, 1974). This can probably be one of the reasons that the sonicated emulsions have higher creaming

stabilities in the lower power range around 10 W, which is apparent in figures 1, 3, 4, 5 and 7. Another feature observed, which probably also can be related to this difference in the particle size distribution between the ultrasonic device and the valve homogenizer, is the smoother curves obtained for the sonicated emulsions compared to the more abruptly changing curves obtained for the valve-homogenized products (see figures 2, 5 and 7).

The increase in creaming stability at higher levels of power input, seen in figures 3 to 6, has also been observed by Walstra (1975) for pasteurized whole milk, when valve homogenized. It is interesting to note that the improvement in creaming stability for the valve homogenized emulsions does not occur until a power level of 10 W has been passed. This is clearly visible in figures 3 to 6.

The time of emulsification, as reflected in an increased number of passes, causes in general an enhanced creaming stability of the emulsions, as can be seen in figures 7 to 9. This is in accordance with Walstra (1975), who demonstrated that on repeated homogenization, the size distribution of milk becomes narrower and globule size smaller. For some of the emulsions viewed in figures 7 to 9, the increase in creaming stability levels off. Gopal (1968) also states that prolonging the emulsification beyond an optimum time interval does little to improve the quality of the emulsions.

If a comparison is performed between the proteins as emulsifiers, the WPC seems to give the best emulsions in terms of creaming stability, which can be judged from figures 4, 6, 8 and 9. This is

especially so for WPC dispersed in distilled water, whereas the addition of NaCl to 0.2 M reduces the creaming stability of the WPC-stabilized emulsions (figures 6 to 9). Another interesting feature of the WPC as an emulsifier is its sensitivity to valve homogenization, which is evident from figures 7 to 9.

The soy protein gives emulsions of relatively high creaming stability mostly in between those stabilized with WPC and sodium caseinate.

The sodium caseinate-stabilized emulsions have the lowest creaming stabilities, especially when the caseinate is dispersed in distilled water. It is interesting to note that the addition of salt up to 0.2 M NaCl in the sodium caseinate stabilized emulsions improves the creaming stability, whereas the reverse is found for the two other proteins studied.

The approach of standardizing the emulsifying procedure and studying its influence seems to be promising as a method for evaluating the emulsifying characteristics of a protein product on a more general basis. But before a more thorough interpretation of the action of proteins as emulsifiers can be given, additional methods of characterizing the emulsions are needed. This is at present under investigation in our laboratories.

ACKNOWLEDGMENT

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REFERENCES

- Gopal, E.S.R. 1968. Emulsion Science, 1 (ed. P. Sherman). Academic Press, London.
- Hermansson, A.-M. 1973. Functional Properties of Proteins for Foods - Solubility. AULs halvårsskrift No. 2, Kemicentrum, Lund, Sweden.
- Mulder H. and Walstra P. 1974. The Milk Fat Globule. Emulsion Science as Applied to Milk Products and Comparable Foods. Pudoc, Wageningen and Commonwealth Agric. Bureaux, Farnham Royal.
- Schmidt, D.G. and Buchheim, W. 1975. Particle Size Distribution in Casein Solutions. Neth. Milk Dairy J. 29: 17.
- Tornberg, E. and Hermansson, A.-M. 1977. Functional Characterization of Protein Stabilized Emulsions: Effect of Processing. J. Food Sci. 42: 468.
- Tornberg, E. and Lundh, G. Functional Characterization of Protein Stabilized Emulsions: Standardized Emulsifying Procedure. In press (J. Food Sci.).
- Walstra, P. 1975. Effect of Homogenization on the Fat Globule Size Distribution in Milk. Neth. Milk Dairy J. 29: 279.
- Walstra, P. and Oortwijn, H. 1975. Effect of Globule Size and Concentration on Creaming in Pasteurized Milk. Neth. Milk Dairy J. 29: 263.

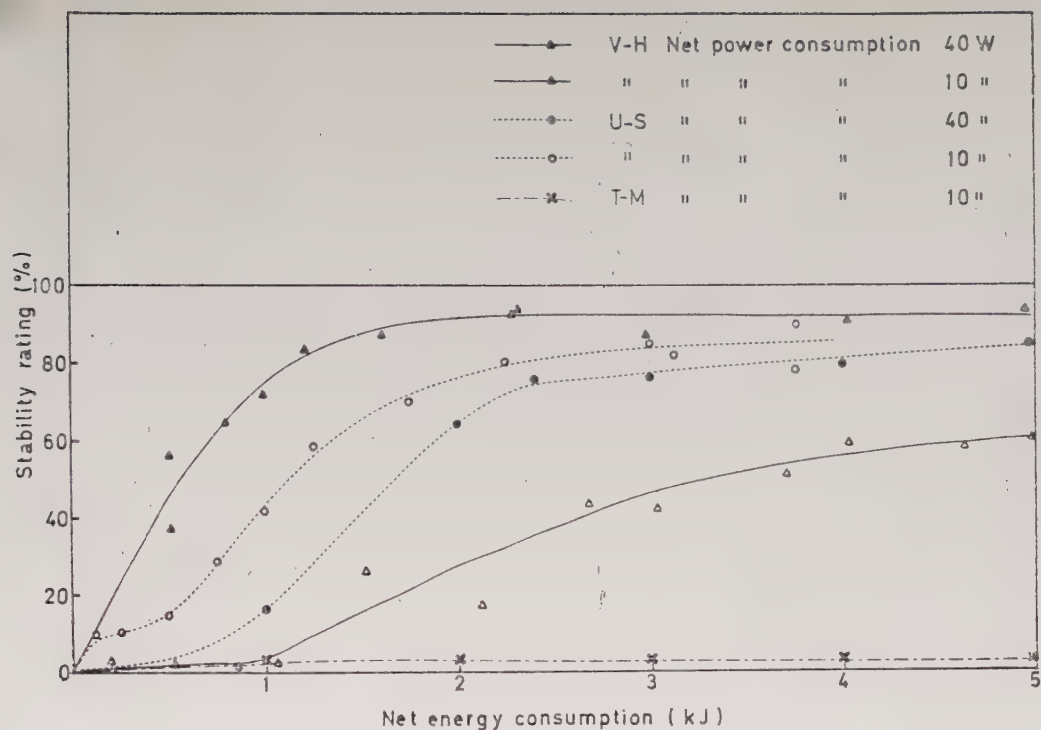


Figure 1. Stability rating of WPC (0.2 - 7) stabilized emulsions as a function of net energy input, on emulsification with a turbo-mixer (T-M), a valve homogenizer (V-H) and an ultrasonic device (U-S) at a power input of 10 and 40 W.

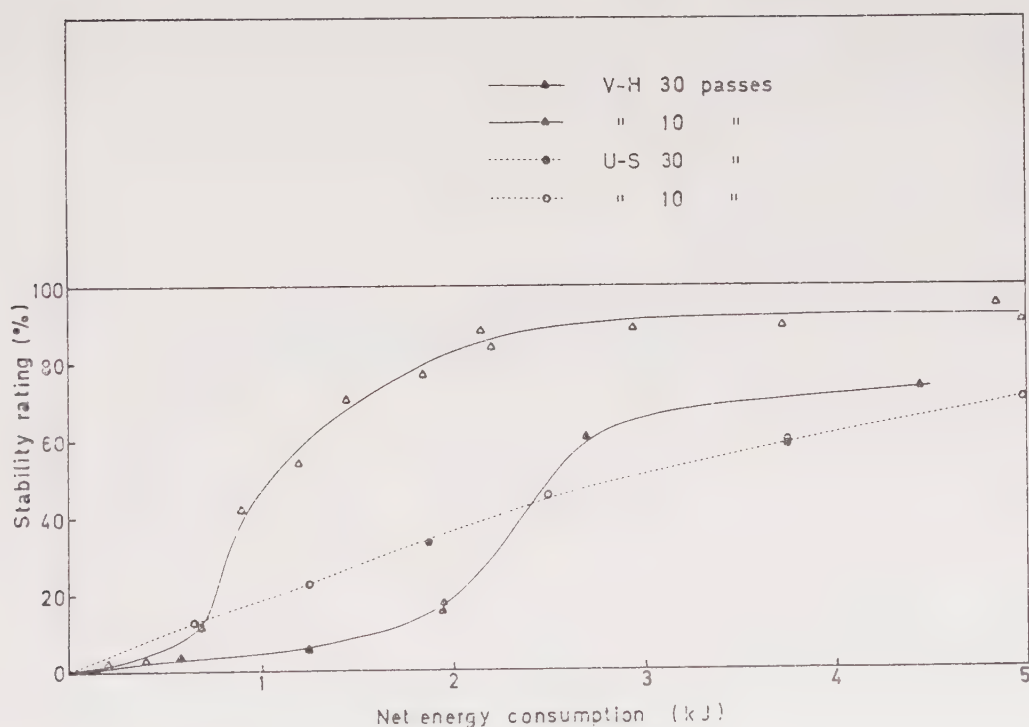


Figure 2. Stability rating of caseinate (0.2 - 7) stabilized emulsions as a function of net energy input, on emulsification with a valve homogenizer (V-H) and an ultrasonic device (U-S) at 10 and 30 passages through the recirculating system.

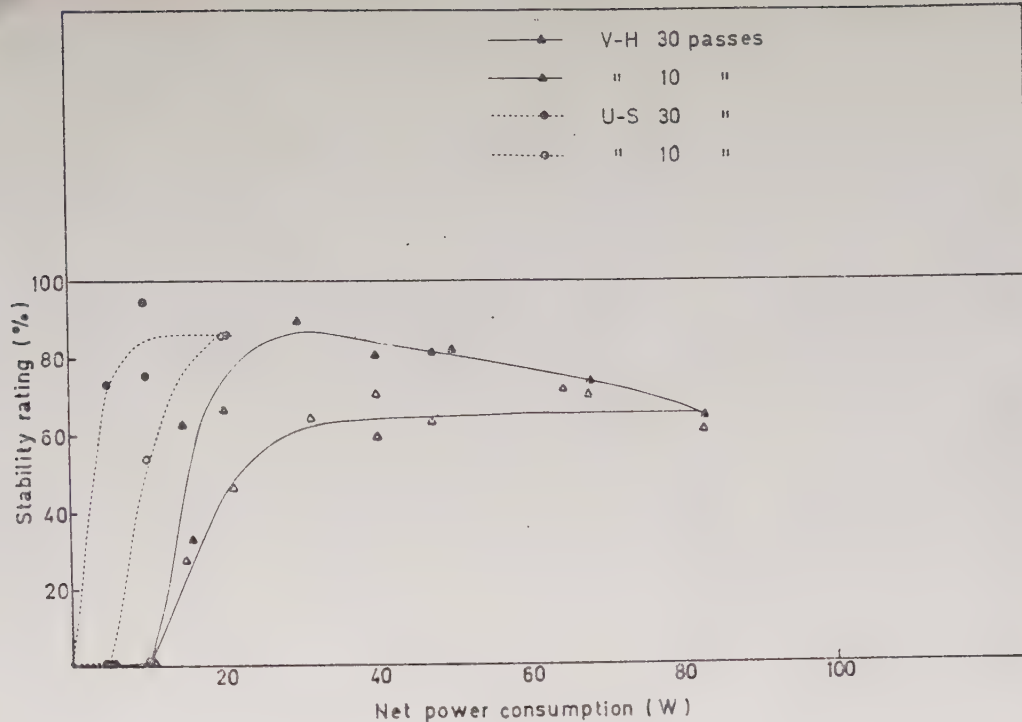


Figure 3. Stability rating of soy protein (0.2 - 7) stabilized emulsions as a function of power consumption, on sonication (U-S) and valve homogenization (V-H) at 10 and 30 passages through the recirculating emulsification system.

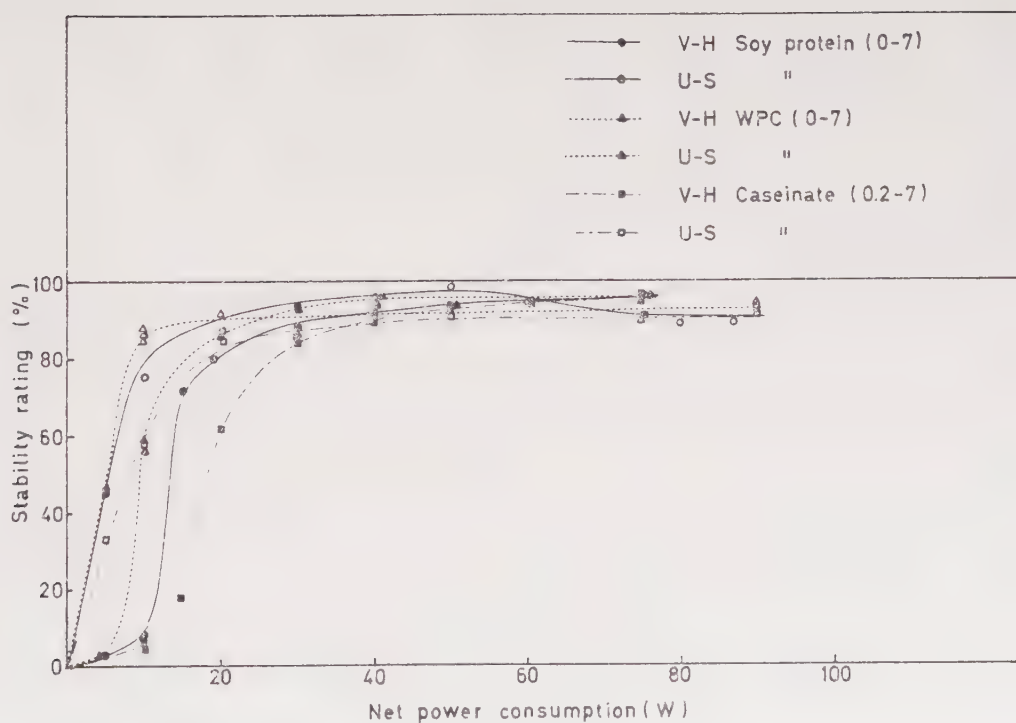


Figure 4. The creaming stability (SR) of soy protein (0 - 7)-, WPC- (0 - 7)- and caseinate (0.2 - 7) stabilized emulsions as a function of power input, on sonication (U-S) and valve homogenization (V-H) at 30 passes through the recirculating system.

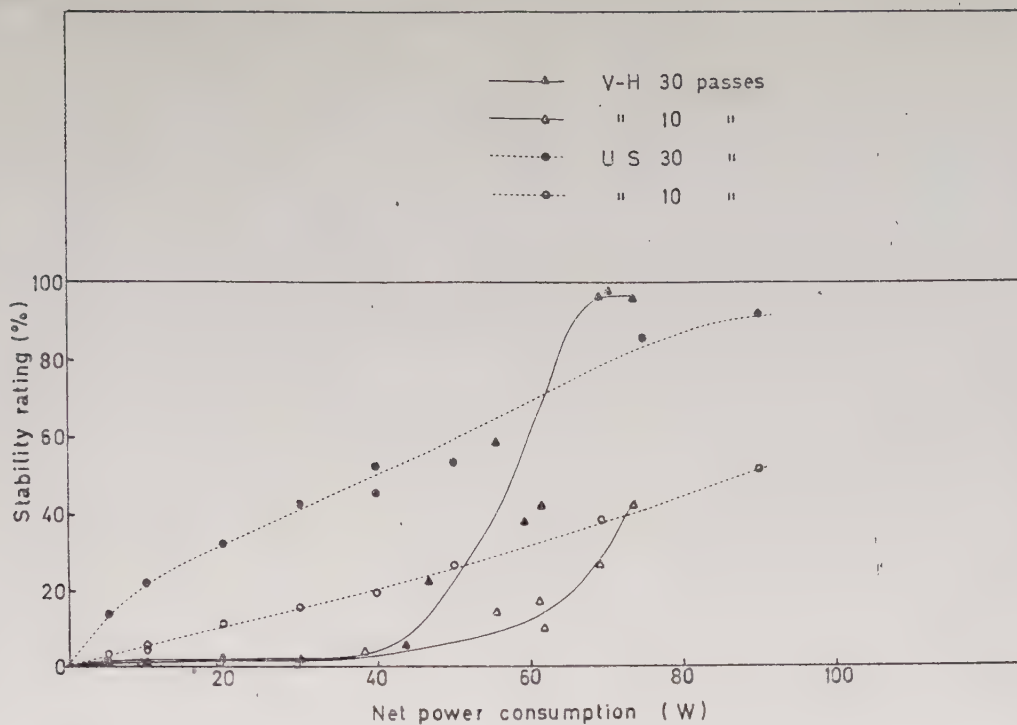


Figure 5. Stability rating of caseinate (0 - 7) stabilized emulsions as a function of power consumption, on sonication (U-S) and valve homogenization (V-H) at 10 and 30 passages, respectively.

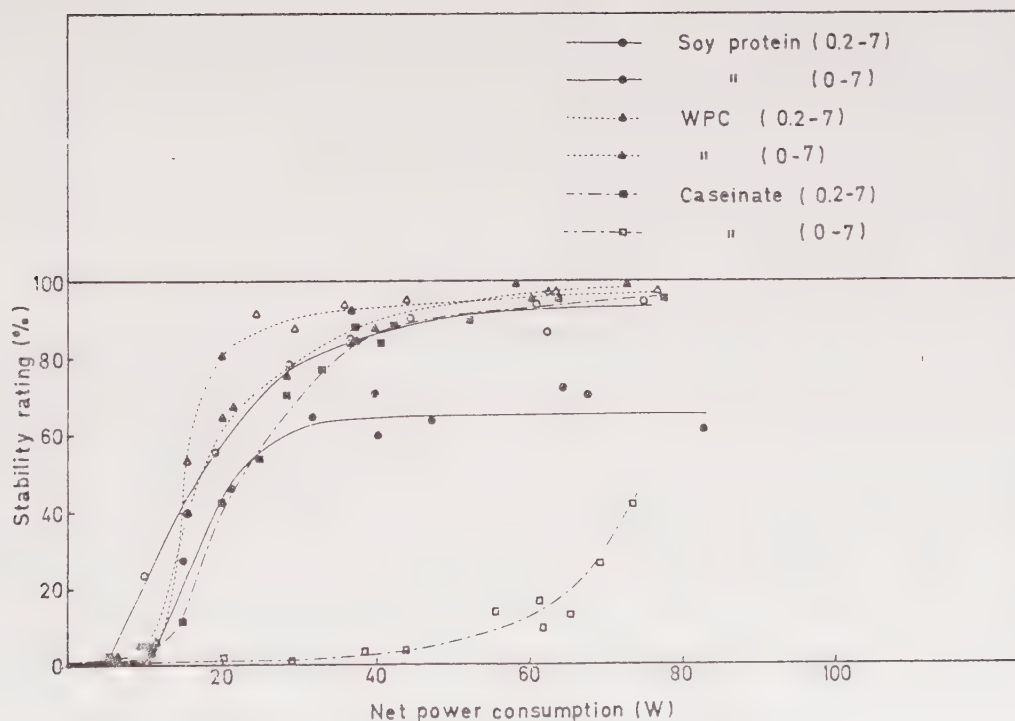


Figure 6. The creaming stability (SR) of soy protein (0.2 - 7)-, soy protein (0 - 7)-, WPC (0.2 - 7), WPC (0 - 7), caseinate (0.2 - 7)- and caseinate (0 - 7) stabilized emulsions as a function of power input, on valve homogenization at 10 passages through the recirculating system..

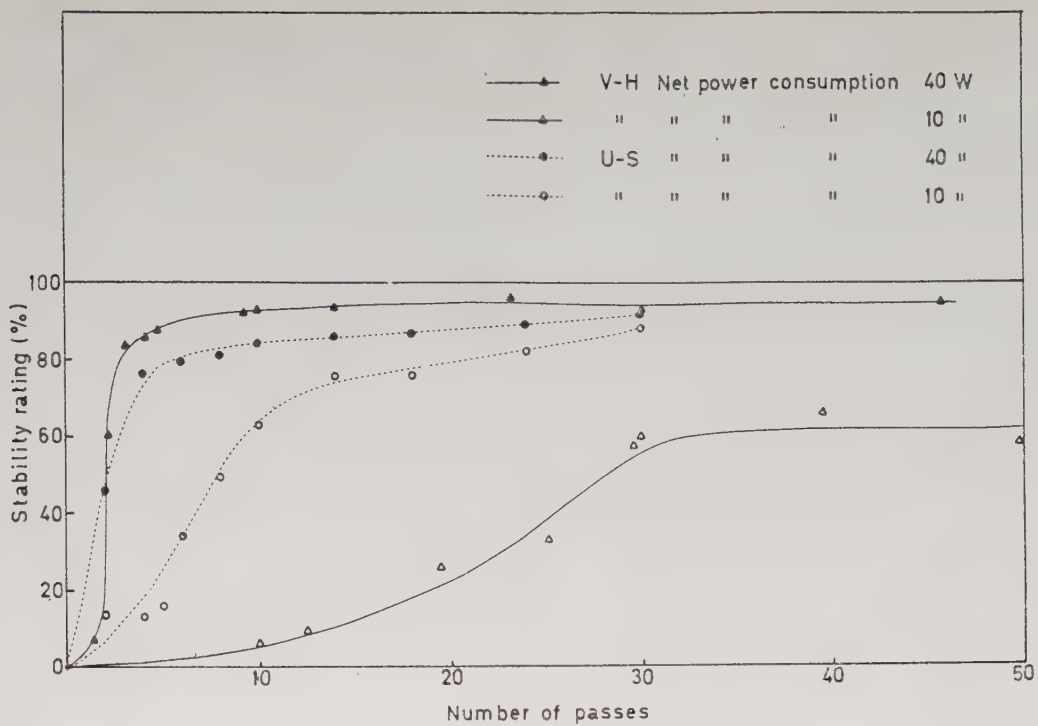


Figure 7. Stability rating of WPC (0 - 7) stabilized emulsions as a function of number of passes, on emulsification with a valve homogenizer (V-H) and an ultrasonic device (U-S) at a power input of 10 and 40 W.

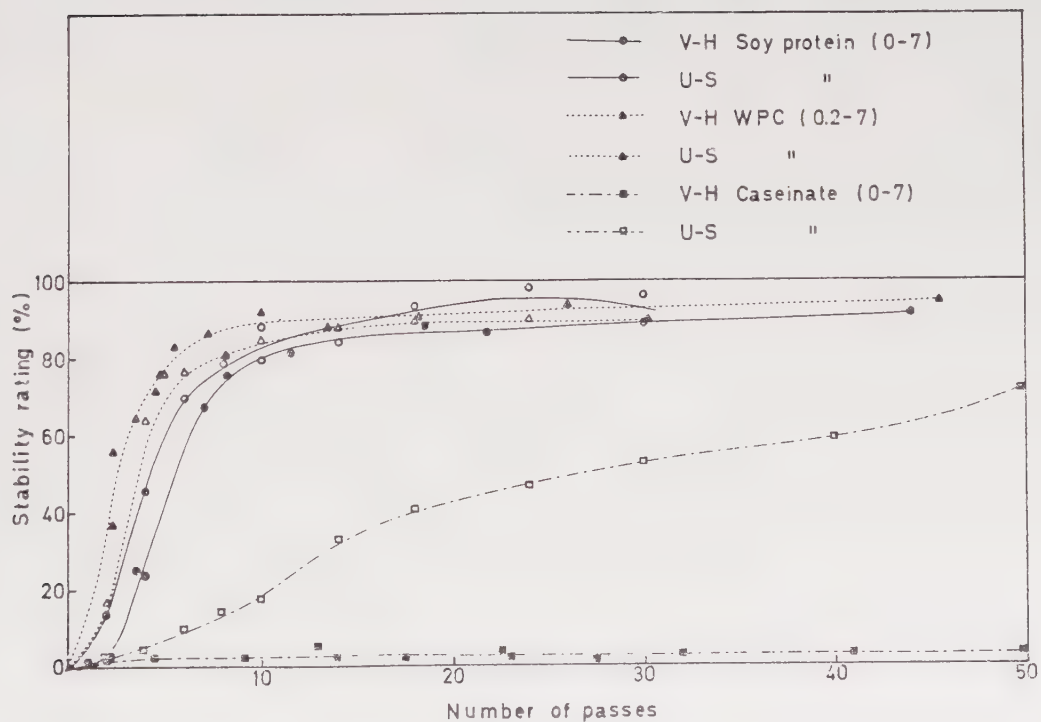


Figure 8. The creaming stability of soy protein (0 - 7)-, WPC (0.2 - 7)- and caseinate (0 - 7) stabilized emulsions as a function of number of passes, on sonication (U-S) and valve homogenization (V-H) at a power input of 40 W.

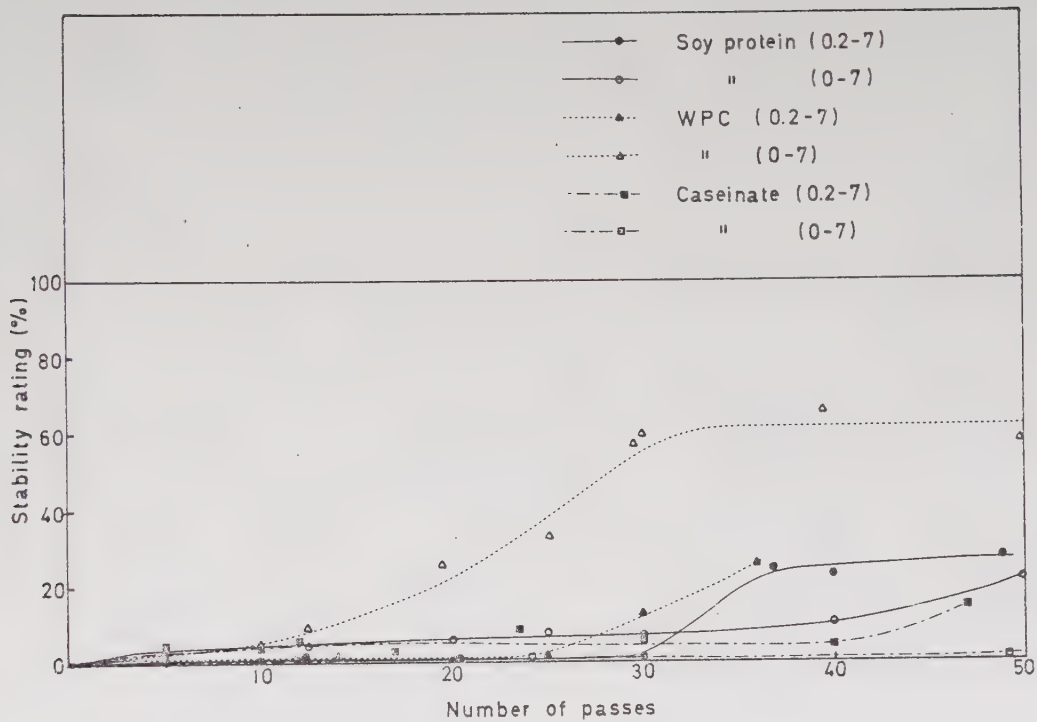


Figure 9. Stability rating of soy protein (0.2 - 7)-, soy protein (0 - 7)-, WPC (0 - 7)-, WPC (0.2 - 7)-, caseinate (0.2 - 7)- and caseinate (0 - 7) stabilized emulsions as a function of number of passes on valve homogenization (V-H) at a power input of 10 W.

FUNCTIONAL CHARACTERIZATION OF PROTEIN STABILIZED EMULSIONS:
EMULSIFYING BEHAVIOUR OF PROTEINS IN A VALVE HOMOGENIZER

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Abstract

Protein stabilized emulsions have been prepared in a valve homogenizer incorporated into a recirculating emulsification system, where power input and number of passes have been varied. The food proteins studied were a soybean protein isolate, a whey protein concentrate (WPC) and a sodium caseinate. The emulsions obtained were characterized in terms of particle size distribution and amount of protein adsorbed on to the fat surface (protein load).

Generally, the final fat surface area of the emulsions obtained increases more as a function of power input than as a function of number of passes. Distribution width, c_s , decreases mostly with increasing power supply and number of passes, but at the highest power input c_s increases. The protein load on the fat globules is largely determined by the fat surface area and by the type of protein adsorbed. The soy proteins give a high protein load and the caseinates give a low protein adsorption at small fat surface areas created. This relation is, though, reversed at large surface areas of the fat globules. The relation between percentage protein adsorbed from bulk as a function of surface area suggests that the caseinates mainly cover the newly created interface by adsorption from the bulk, whereas the soy proteins fulfil this task mostly by spreading at the interface. Salt addition to 0.2 M

NaCl enhances protein adsorption at the fat globule interface in the case of soy protein and caseinate, but for the whey proteins protein load is higher in distilled water.

Introduction

The study of emulsifying properties of proteins has evolved via two main approaches: emulsifying capacity and emulsion stability measurements. The former has merely been used as a crude test in connection with functional characterization of various plant and animal protein products, whereas the latter, defined as coalescence stability, has been the subject of a considerable amount of basic research. As protein stabilized emulsions are usually very stable and the adsorption of proteins at interfaces can be considered as mainly irreversible, the emulsifying properties of the proteins during the emulsification process become increasingly important in determining the properties of the emulsion formed. This has been realized in the literature of functional properties of proteins by Tornberg and Hermansson¹ and Tornberg², and for other polymer stabilized emulsions by Lankveld³ and Böhm⁴ and for milk products by Henstra and Schmidt⁵, van Ogden et al.⁶ and Todt⁷.

With the approach of studying the emulsifying properties of proteins as a function of emulsification variables, the emulsification process has to be standardized and quantified. This has been achieved by using a recirculating system, where the flow velocity, power and energy input are controlled and measured. This emulsification system has been described elsewhere⁸. It has been used in a study of the creaming stability of protein stabilized emulsions, which were differently made in a turbo-mixer, a valve homogenizer and an ultrasonic device². Because there are many factors affecting

the creaming behaviour of the emulsions formed, additional methods of characterizing the emulsions are needed, to be able to deal with the emulsifying characteristics of proteins more thoroughly. The aim of this article is to study the particle size distribution and the amount of protein adsorbed on to the fat surface (protein load) of protein stabilized emulsions as a function of power input and number of passes. The emulsions are made with a valve homogenizer incorporated into a recirculating system as described earlier⁸. Three protein products have been used as emulsion stabilizers; a soy protein isolate, a sodium caseinate and a whey protein concentrate (WPC).

Experimental

2.1 Materials

Soy protein isolate. A soy protein isolate produced under mild conditions was kindly provided by Central Soya. It was prepared from defatted soybean flakes by extraction with deionized water, precipitation at pH 4.6, washing the curd with deionized water, neutralizing to pH 7.0 and spray drying. Analysis: protein (N x 6.25) 96.1% (dry weight); solubility determined according to Hermansson⁹ in distilled water and in 0.2 M sodium chloride solution at pH 7, denoted as (0 - 7) and (0.2 - 7), is 97.3% and 74.1%, respectively.

Caseinate. Spray blend caseinate (DMV, Holland) a commercially available sodium caseinate, was used. Analysis: protein (N x 6.43) 93.4% (dry weight), solubility determined as above in distilled water and in 0.2 M sodium chloride solution at pH 7, denoted as (0 - 7) and (0.2 - 7), is 100% in both cases.

Whey protein concentrate (WPC). The whey protein concentrate was obtained by ultrafiltration (UF) and spray drying of cheese whey. UF was carried out at 5°C in a pilot plant unit from De Danske Sukkerfabriker (DDS) with a membrane area of 2.2 m² and with a membrane type designated as 600 DDS. Analysis: protein (N x 6.55) 69.8% (dry weight); solubility determined as above in distilled water and in 0.2 M sodium chloride solution at pH 7, denoted as (0 - 7) and (0.2 - 7), is 94.1% and 94.9%, respectively.

Soybean oil. A commercially available soybean oil (AB Karlshamns Oljefabriker, Karlshamn, Sweden) was used. Analysis: fatty acid composition 18:2 54%, 18:1 23%, 16:0 10%.

2.2 Preparation of samples

Protein dispersions (2.5% (w/w), based on the protein content) in distilled water or sodium chloride solution were made with the Sorvall omni-mixer. The pH was adjusted with 0.2 M NaOH or 0.2 M HCl. Soybean oil was added directly to the protein dispersion to attain 40% oil by weight.

2.3 Emulsion formation

A quantity of 50 grams of emulsion was emulsified in a recirculating system as previously described⁸. The description of the emulsifying apparatus and the measurement of the power and energy input have been given in the same paper. The flow rate of the emulsion through the recirculating system was held constant at $250 \pm 25 \text{ ml min}^{-1}$, giving an average residence time of an emulsion droplet during one passage, t_p , of $12.5 \pm 0.4 \text{ s}$, and an average stroke rate of 0.9 stroke/s in the valve homogenizer.

The system was cooled during the emulsification procedure to keep the temperature of the emulsion at $25^{\circ}\text{C} \pm 2$ during processing.

2.4 Particle size determination

Accurate determinations of the particle size distribution in emulsions cause many problems especially of the particles in the submicron range. Microscopic and sedimentations methods and also the Coulter Counter method have been applied, but the spectro-turbidimetric method was preferred in this study as it offers a rapid, easy and reproducible method of analysis. This method has been applied in milk research^{10,11,12}, and has also been found suitable for paraffin-water emulsions^{12, 3, 4}. The method is fully described elsewhere^{11,12}.

The optical density, E , of the diluted emulsions is measured as a function of the wavelength, λ , varied between 0.38 and 1.70μ . With these data and the knowledge of the volume fraction of the disperse phase (ϕ), optical path length (l) and the refractive indices of the continuous (n_2) and the disperse phase (n_1) the reduced turbidity (Z), can be calculated. Z is plotted against $\log \left[\frac{2 \pi (n_1 - n_2)^2}{\lambda} \right]$, and the experimentally derived curve is compared with theoretical spectra, representing a wide range of size distributions, being log normal and truncated log normal. The theoretical curve of best fit gives a good approximation of the spread, and the logarithm of the average globule size is given directly by the shift of abscissa. The goodness of fit has been ranked in a scale from 1 to 10 and the average value obtained is 6.9 ± 2.1 for all the emulsions studied. The curves which could not be fitted to any theoretical spectrum, formed, on average, 5% of the total, except for soy protein (0.2 - 7) stabilized emulsions, where 20% could not be fitted to any theoretical curve, mainly those valve

homogenized emulsions using the low power range.

The experiments were carried out with a Zeiss PMQ II spectrophotometer, for which an attachment for turbidimetric analysis is available. The apparatus has been modified according to Walstra¹¹ to give an angle of acceptance of 1.5 degrees in water. At such a small angle of acceptance, the forward scattering can be corrected for¹³, and multiple scattering can be neglected, as long as the optical density of the diluted emulsions lies between 0.15 and 0.7¹³. Dependent scattering was avoided by using optical cells of 5 mm path length and diluting the emulsions in water so that they would never exceed 0.0004 in volume fat fraction (cf. ref.¹³). To minimize the influence of the band width, the slit width was kept low, never exceeding 0.08 mm at the highest wavelength. The two cells used were checked against each other, giving a difference in adsorbance of ± 0.001 , which can be considered to lie within the limits of error. As a reference for each measurement, the diluted emulsion filtered through a Millipore filter (GSPW 0.22 μ) was used.

The refractive index of soybean oil at room temperature and at every wavelength were determined by the method described by Walstra¹². The values of n_2 for pure water at 20°C were used. It should also be mentioned that Walstra¹² has shown that the milk fat globules in scattering experiments show the same refractive index as does the separated fat in bulk.

Before measuring the optical density of the diluted emulsions the aggregates must be disintegrated into single globules. Because the aggregation of the emulsions formed depends on the protein used as emulsifier and on the emulsification conditions, the

disintegrating agent has to be found individually for every protein stabilized emulsion made in different ways in the valve homogenizer. For both the caseinates, i.e. caseinate (0.2 - 7) and caseinate (0 - 7), it was sufficient to dilute the emulsions according to the recommended concentrations of solution A as a function of the concentration of disperse phase, according to Walstra¹¹. Furthermore the optical density of a caseinate (0.2 - 7) stabilized emulsion was plotted against the volume fraction (ϕ) of the dispersed phase, and a linear relationship between the optical density and ϕ at every wavelength was obtained, which implies that multiple scattering is absent, and that no aggregation or dispersion occurs upon dilution. The WPC (0 - 7) stabilized emulsions needed a concentration of the dispersing agent twice that of solution A, whereas the more aggregated WPC (0.2 - 7) stabilized emulsions needed ten times. The heavily aggregated soy protein stabilized emulsions, as revealed by the microscope, was dispersed in a solution of SDS to a final concentration of 0.5%. This dispersing solution caused complete disintegration, as checked by microscope and by the turbidity of the diluted emulsions with different amounts of the dispersing agent. The last check revealed if any coalescence occurred due to the presence of the dispersing agent; no coalescence was observed. It was also found for all the protein stabilized emulsions studied that changing the power input or number of passes during homogenization of the emulsions did not affect the effectiveness of the chosen dispersing solutions.

The average globule size obtained in the spectroturbidimetric method is the arithmetic mean of the surface-weighted distribution, denoted as d_{VS} . This mean value is directly related

to the specific surface area, A , by the following formula:

$$A = \frac{6 \Phi}{d_{vs}} \quad \text{m}^2 \text{ fat/ml emulsion}$$

The spread of the size distribution is given as c_s , which is the relative standard deviation of the surface-weighted distribution.

The reproducibility of the measurements is good (1% or better), and the major source of error lies in the difficulty of fitting the theoretical spectra to the experimental data, especially if d_{vs} is very small¹⁵.

2.5 Protein load determination

Methods of determining the amount of protein adsorbed per unit area of interface are based on separation of the two dispersed phases, whereafter the protein content in the water phase⁶ or on the oil phase¹⁶ or in both¹⁴ is measured. The interfacial area is derived from the particle size distribution according to the calculations described above.

The separation technique which is most suitable depends on the properties of the emulsion. Centrifugation at high speed, leading to a water phase almost free from globular fat, is straight forward, if the emulsion is coalescence stable, free from very small globules and if no sedimentation of protein particles from the water phase occurs. If these prerequisites are not met, it is possible to centrifuge at a lower speed to get rid of most of the fat globules, after which the rest of the disperse phase in the water phase is filtered off.

The following procedure was worked out for the present emulsions. All the protein stabilized emulsions were centrifuged at 40 000 x g for 30 minutes, except for the soy protein (0.2 - 7) stabilized emulsions, which were centrifuged at the lower speed of 10 000 x g. The lower water phase obtained after centrifugation was sucked off with a syringe. It was compared in adsorbance with the original protein solution centrifuged at the same speed and of the same protein content. If the lower water enriched phase was more turbid than the original protein solution, this was taken as an indication of fat particles occurring in the water phase. In that case, the water enriched phase was diluted 30 times, and then filtered through a Millipore filter (GSWP, 0.22 μ). The determination of the protein content in the aqueous phase was made by analysing for nitrogen by the general Kjeldahl procedure with an accuracy of $\pm$ 0.5%.

Coalescence of fat globules, obvious as free fat, was not observed after centrifugation for any of the protein stabilized emulsions. To check whether centrifugation leads to desorption of any membrane material and/or to sedimentation of protein particles, the emulsions were centrifuged at two speeds, 10 000 x g and 40 000 x g, and the percentage adsorption on the fat surface area was compared. If there was any difference as a function of the increased centrifugation force, this was due to sedimentation of protein particles rather than removal of protein from the surface layer. The maximum error introduced by sedimentation of the proteins during centrifugation can be read off from the solubility data given in section 2.1, because the solubility of the protein is determined after centrifugation of the protein dispersion at 40 000 x g for 30 minutes⁹. For both the caseinates no sedimentation occurs, as the solubility

is 100%. In the case of WPC (0.2 - 7) and WPC (0 - 7) the maximum error is 5 - 6%, but this number is probably reduced by more than half for the WPC stabilized emulsions, as visually judged from the amount of sediment occurring. Sedimentation can at most be 2 - 3% for soy protein (0 - 7) stabilized emulsions, but in the case of soy protein (0.2 - 7) this affects the result more strongly, as only 82.4% of the protein remains soluble after centrifugation at $10\,000 \times g$ for 30 minutes. This number of 17 - 18% sedimentation is very much reduced when centrifuging the emulsions, because it has been shown by Farnum et al.¹⁷ that the IIS fraction, the high molecular weight fraction of soy proteins, is mainly incorporated in soy film formation.

Filtration of the lower water phase, in the case of fat globules which have not been centrifuged off, can introduce errors such as protein retention in the filter. This error can be minimized by diluting the sample, and pre-experiments showed that 30 times dilution was enough in all cases. To estimate the maximum error, the protein retention was measured for protein dispersions of 2.5% protein content, which were centrifuged, diluted 30 times and filtered. The retention could vary from 1 to 5%, due to the protein used. The values for the emulsions are probably much lower, as for example indicated by a reduced need for the application of pressure during filtration of the emulsions as compared to the protein solutions.

Results and discussion

3.1 Fat surface area created as a function of power input and number of passes

In figure 1 the fat surface area obtained at 10 passes is plotted against power input during valve homogenization for all the protein stabilized emulsions. The almost linear curves up to 40 W coincide within the limits of error for all the emulsions, except for the one stabilized with soy protein (0 - 7), which has a stronger dependence of power input up to 25 W, after which a rather labile plateau is obtained between 25 and 40 W. The divergence between the protein stabilized emulsions observed at higher power inputs than 40 W, shows that the emulsions of smallest globule size and largest surface areas are those stabilized by soy protein (0 - 7), followed by caseinate (0.2 - 7), WPC (0 - 7) and caseinate (0 - 7). The surface area of the three last mentioned emulsions more or less continue the linear dependence on power input. The curves obtained for WPC (0.2 - 7) and soy protein (0.2 - 7) adopt a more sigmoidal behaviour, with the latter giving emulsions with the largest globules and therefore the smallest fat surface area. It is interesting to compare the fat surface area obtained with this small-scale valve homogenizer with one (flat-valve type) homogenizing milk of 37% fat at a flow rate of 100 l/h. The fat surface area obtained by Goulden and Phipps¹⁸ with such a homogenizer at a pressure drop of 100 kg f/cm² (≈ 80 W) was ≈ 2.4 m²/ml emulsion.

Figure 2 gives the fat surface area of all the protein stabilized emulsions as a function of the number of passes at a power consumption of 40 W. For the WPC (0.2 - 7) and caseinate (0.2 - 7) stabilized emulsions there is a weak and almost similar dependence on the number of passes. Caseinate (0 - 7) gives rise to emulsion

with somewhat larger surface areas than the two forementioned, but still the increase in surface area is very small, as compared to the enlargement of surface area due to power input. The WPC (0 - 7) stabilized emulsions are, however, more sensitive to the number of passes, and areas as large as $2.5 \text{ m}^2/\text{ml}$ can be obtained at 20 passages or more. The soy protein stabilized emulsions behave somewhat differently, both having a rather wide scatter in results, which is especially pronounced for soy protein (0.2 - 7). There is a slight overprocessing in terms of fat surface area for the soy protein (0 - 7) stabilized emulsions after 25 passages. It is interesting to note the large surface areas that can be made for the soy protein (0.2 - 7) stabilized emulsions as a function of number of passes ($\approx 3.5 \text{ m}^2/\text{ml}$), as compared to those obtained with increasing power input, not exceeding $3 \text{ m}^2/\text{ml}$. This is the opposite behaviour compared to the other proteins, where an increase in power consumption favours the production of small fat globules.

3.2 Size distribution width as a function of power input and number of passes

The relative standard deviation, c_s , for the surface-weighted distribution is given as a function of the net power consumption in figure 3 at a constant number of passes (10). Both soy protein (0.2 - 7) and soy protein (0 - 7) give rise to emulsions with larger spread than the other proteins, which is most pronounced at higher levels of power input. Every curve in figure 3 shows a minimum in c_s in the medium range of power input, whereafter c_s increases again at the highest power consumptions. This minimum in c_s is rather flat ranging from 30 to 60 W except for the soy protein (0 - 7) stabilized emulsions, where

the minimum in c_s is between 20 and 40 W and the rise in c_s at higher power inputs than 40 W is steep, reaching values of c_s as high as 2.0 at 85 - 90 W. The steep rise in c_s for the soy protein (0 - 7) stabilized emulsions above 40 W occurs simultaneously as large fat surface areas are formed (see figure 1). The caseinate (0.2 - 7), WPC (0 - 7) and caseinate (0 - 7) stabilized emulsions have a rather similar surface area - power dependence, as revealed by figure 1, which is also the case concerning the power dependence of c_s . Valve homogenizing milk gives a different behaviour because distribution width sharply increases with pressure and reaches a maximum before reaching a steady value¹⁹.

In figure 4, c_s is plotted as a function of number of passes at a constant power input of 40 W for all the protein stabilized emulsions. For the WPC and caseinate stabilized emulsions the size distribution becomes narrower for increased number of passes, which is in agreement with the results obtained for milk¹⁴.

In comparison with figure 3 it can be seen that the narrowest size distribution ($c_s \approx 0.3$) can be obtained by emulsifying at a high number of passes. This does not hold for the soy protein (0.2 - 7) stabilized emulsions, which get larger distribution widths for an increased number of passes. The wide scatter in results for the soy protein (0.2 - 7) stabilized emulsions is very pronounced, as also evident in figure 2. The curves of the WPC (0.2 - 7), caseinate (0.2 - 7) and caseinate (0 - 7) stabilized emulsions more or less coincide in figure 4, but the curve of the WPC (0 - 7) stabilized emulsions is above the other three. This relationship is also observed in figure 2.

3.3 Protein load_and_percentage adsorbed_as_a_function_of_fat surface_area

The amount of protein adsorbed in mg per m^2 fat surface area and the percentage adsorption from bulk are plotted against fat surface area created in figures 5 and 6 respectively for all the protein stabilized emulsions. The number of passes has been held constant at 10 and the surface area increase has been obtained by augmenting the power input in accordance with figure 1. The most striking feature to be observed in figures 5 and 6 is the very different behaviour shown by the caseinates as compared to the soy proteins. The emulsions stabilized by the latter have a very high protein load at small surface areas, as can be seen in figure 5, whereafter it diminishes almost exponentially as the surface area expands. The caseinates, though, give low protein coverage for emulsions of small surface area, but the protein load grows as a function of the surface area to a maximum at about $1.5 \text{ m}^2/\text{ml}$ emulsion, after which it decreases slightly. The different behaviour shown by the two proteins corresponds in figure 6 to a higher percentage adsorption by the soy proteins at small surface areas and a lower percentage adsorption at the larger surface areas as compared to the caseinates. To cover the interface with protein at an enlarged surface area the caseinates especially in 0.2 M NaCl, supply protein mainly from the bulk, as revealed from figure 6. The soy proteins, though, supply protein from bulk to a lesser extent especially for areas larger than $2.0 \text{ m}^2/\text{ml}$, where percentage adsorption is more or less linear as a function of surface area. This indicates that the newly created interface is mostly covered by spreading or unfolding of the already adsorbed soy protein molecules at the interface. Emulsions of surface area exceeding 2 m^2 fat/ml emulsion evidently

will have a higher protein load when stabilized by caseinate than by soy protein, when compared at the same ionic strength. For both proteins salt addition induces more protein to be adsorbed at the interface for all the surface areas investigated, which is confirmed both in figures 5 and 6. In this connection it can be interesting to compare the values obtained by Graham¹⁶ for the maximum amount of irreversibly adsorbed β -casein (pH = 7; ionic strength = 0.1) at the air-water interface, which comprises the primary layers of molecules. His data of 2.7 mg/m^2 is in between those obtained for caseinate (0.2 - 7) and caseinate (0 - 7) at surface areas larger than $1.0 \text{ m}^2/\text{ml}$, which suggests that the protein load under these conditions is composed of a monolayer, being more densely packed at the higher ionic strength.

The whey proteins behave in a manner intermediate between those of the two other proteins. The abrupt decrease in protein load to a surface area of $0.6 \text{ m}^2/\text{ml}$ observed for the WPC (0 - 7) stabilized emulsions corresponds to a decrease also in percentage adsorption, which may be interpreted as desorption taking place in this range as a function of increased surface area generated. At larger areas than $0.6 \text{ m}^2/\text{ml}$ a steady increase in percentage adsorption occurs for the WPC (0 - 7) stabilized emulsions up to $1.5 \text{ m}^2/\text{ml}$, which corresponds to a faint maximum in protein coverage, which can be seen in figure 5. Then spreading of already adsorbed WPC (0 - 7) molecules dominates up to a surface area of $2.8 \text{ m}^2/\text{ml}$, whereafter a change of the dominant mechanism for covering the interface occurs once more to supply from bulk, which gives a small increase in protein load at the largest surface areas created. The WPC (0.2 - 7) stabilized emulsions have the lowest protein coverage ($\approx 2 \text{ mg/m}^2$) at fat surface areas between 1.0 and $2.0 \text{ m}^2/\text{ml}$ of all the protein stabilized emulsions studied, whereas at larger surface areas the soy

those stabilized with WPC (0.2 - 7). It is interesting to note that an increase in ionic strength to 0.2 M NaCl does not increase the amount of protein adsorbed for the whey proteins, but rather the other way round, which is opposite to the behaviour of the other two proteins. The observed maximum in protein load for emulsions stabilized with WPC (0.2 - 7) in figure 5 at a fat surface area of $\approx 0.6 \text{ m}^2/\text{ml}$ corresponds to a kink in the curve for WPC (0.2 - 7) in figure 6. Evidently the supply from bulk is more pronounced at small fat surface areas, whereas spreading of already adsorbed WPC (0.2 - 7) molecules grows more important for larger surface areas.

Figures 5 and 6 give clear evidence that not only the size of the fat globules and thereby the fat surface area, but also the amount of interfacial film is strongly affected by the way the emulsions are made, which in turn influences the properties of the emulsions formed. It has been observed for milk products that when the homogenization intensity is increased the protein load on the fat globules is enhanced^{5,6,7}.

The behaviour at an air-water interface of the three proteins investigated here has been studied in an earlier paper²⁰. Although the interfacial studies were performed at the air-water interface and not the oil-water interface, the results will at least provide an indication of the interfacial behaviour. Graham¹⁶ has shown that the adsorption and surface pressure isotherms obtained for β -casein and BSA at the air-water interface are similar to those obtained at the oil-water interface. To allow comparison of the interfacial results with those obtained in this emulsification study, the amount of protein available in the bulk before adsorption in relation to the

interface created are the same in both studies. This calculation procedure gave rise to the relation visible in figure 7, where the interfacial area created, when making emulsions, is plotted against the initial subphase concentration (wt. %) in the interfacial tension study. It can be seen in the figure that the approximate upper limit of the interfacial area of the emulsions of $4.0 \text{ m}^2/\text{ml}$ corresponds to an initial subphase concentration of $5 \cdot 10^{-3}$ wt. % in the interfacial tension measurements.

The curves in figures 5 and 6 seem to confirm the interfacial behaviour of the caseinates and the soy proteins proposed on the basis of the interfacial tension measurements. These measurements implied that although both the caseinates and the soy proteins have a complex quaternary structure, the migration of the molecules to the interface is accomplished in the case of caseinates by the monomers and for the soy proteins by the gross associated complex. Therefore the protein load is very high for the soy proteins at small surface areas, and low for the caseinates, as seen in figure 5. But as the coverage of the interface is mainly achieved through spreading of the soy proteins, as seen from figure 6 and suggested by the interfacial tension results, protein load diminishes very much at larger fat surface areas. The caseinates, on the other hand, supply the created interface with molecules from the bulk even at large surface areas, which is especially evident for caseinate (0.2 - 7). This behaviour might be deduced from the interfacial tension measurements by the fact that diffusion is the rate-controlling step to a high Π . The increased adsorption of the caseinate when salt is added might also be expected from the interfacial tension results, giving a higher diffusion rate and a lower

adsorption barrier for caseinate (0.2 - 7) compared to caseinate (0 - 7). According to Wolf²¹ and Koshiyama²² association and aggregation of the 7S and 11S globulins of the soy proteins is favoured at 0.1 - 0.2 M NaCl, which may explain the higher protein load observed for soy protein (0.2 - 7) when compared to soy protein (0 - 7).

The emulsifying behaviour of the whey proteins, seen in figures 5 and 6, is not as easily correlated with the interfacial behaviour as for the two other proteins. The interfacial data showed that WPC (0 - 7) spreads relatively easily on the interface in relation to WPC (0.2 - 7). But in figure 6 the supply of molecules from bulk by WPC (0 - 7) exceeds that of WPC (0.2 - 7), which gives rise to a higher protein coverage per unit area (see figure 5). This seems to be contradictory, but may be explained if the unfolding of the WPC (0 - 7) molecules at the surface gives rise to enhanced exposing of hydrophobic groups, which induce further adsorption of protein from bulk and hence multilayer formation. Further evidence of this phenomenon is the peculiar increase in percentage adsorption of WPC (0 - 7) molecules at large surface areas, which follows a stage of spreading. The very high protein load of WPC (0 - 7) at small surface areas must indicate the formation of multimolecular layer, because whey proteins are small molecular complexes unable to form such a thick protein layer without multilayer formation.

In figures 8 and 9 the same type of curves have been constructed as those in figures 5 and 6, but in this case the enlarged fat surface area is due to an increase in number of passes in accordance with figure 2. For the soy protein (0.2 - 7) stabilized emulsions the curves in figures 8 and 9 agree well with

those in figures 5 and 6, which show that the protein load and the percentage adsorption will be the same for the same fat surface area, irrespective of how this area has been created. Both the caseinates show a stronger dependence in both protein load and percentage adsorption on the fat surface area, when it has been created by an increased number of passes rather than by increased power supply. This manifests itself in a higher protein coverage for caseinate (0 - 7), but a lower protein load for caseinate (0.2 - 7) at the same fat surface area, when homogenized at 40 W for different numbers of passes. The curves of the WPC stabilized emulsions in figures 8 and 9 are rather similar to those in figures 5 and 6 with minor deviations such as somewhat lower and higher protein coverage for WPC (0 - 7) and WPC (0.2 - 7) stabilized emulsions, respectively, made with an increased number of passes. The supply of protein molecules from bulk is also enhanced in the case of emulsions stabilized by soy protein (0 - 7), as it is for the caseinates, when comparing figure 9 with figure 6. This gives rise to a slightly higher protein load, compared at the same fat surface area for the soy protein (0 - 7) stabilized emulsions, when the fat surface areas are generated by variation in number of passes instead of power supply.

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References

1. Tornberg, E.; Hermansson, A.-M. J. Food Sci. 1977, 42, 468.
2. Tornberg, E. Accepted for publication in J. Food Sci.
3. Lankveld, J.M.C. Thesis. Meded. Landbouwhogeschool Wageningen 70-21 (1970).
4. Böhm, J.Th.C. Thesis. Meded. Landbouwhogeschool Wageningen 74-5 (1974).
5. Henstra, S.; Schmidt, D.G. Neth. Milk Dairy J. 1970, 24, 45.
6. van Ogden, L.; Walstra, P.; Morris, H.A. J. Dairy Sci. 1977, 59, 1727.
7. Todt, K. Milchwissenschaft 1976, 31, 83.
8. Tornberg, E.; Lundh, G. Accepted for publication in J. Food Sci.
9. Hermansson, A.-M. Functional Properties of Proteins for Foods - Solubility. AULs halvårsskrift No. 2, Kemicentrum, Lund, Sweden, 1973.
10. Goulden, J.D.S. Trans. Faraday Soc. 1958, 54, 941.
11. Walstra, P. Neth. Milk Dairy J. 1965, 19, 93.
12. Walstra, P. J. Colloid Interface Sci. 1968, 27, 493.
13. Walstra, P. Brit. J. Appl. Phys. 1965, 6, 1187.
14. Mulder, H.; Walstra, P. 1974. The Milk Fat Globule. Emulsion Science as Applied to Milk Products and Comparable Foods. Pudoc, Wageningen and Commonwealth Agric. Bureaux, Farnham Royal.
15. Walstra, P.; Oortwijn, H.; de Graf, J.F. Neth. Milk Dairy J. 1969, 23, 12.
16. Graham, D.E. 1976. Thesis entitled "Structure of adsorbed protein films and stability of protein stabilized foams and emulsions". Unilever Research Laboratory, Colworth/Welwyn, The Frythe, Welwyn, Hertfordshire.

17. Farnum, D.; Stanley, D.W.; Gray, J.I. Can. Inst. Food Sci. Technol. 1976, 9, 201.
18. Goulden, J.D.S.; Phipps, L.W. J. Dairy Res. 1964, 31, 195.
19. Walstra, P. Neth. Milk Dairy J. 1975, 29, 279.
20. Tornberg, E. Submitted for publication in J. Fd Sci. Agric.
21. Wolf, W.J. In "Soybeans: Chemistry and Technology" (Smith, A.K.; Circle, S.J., eds.), 1972, p. 93. Avi Publishing Company Inc., Westport, Connecticut.
22. Koshiyama, I. Agr. Biol. Chem. 1968, 32, 879.

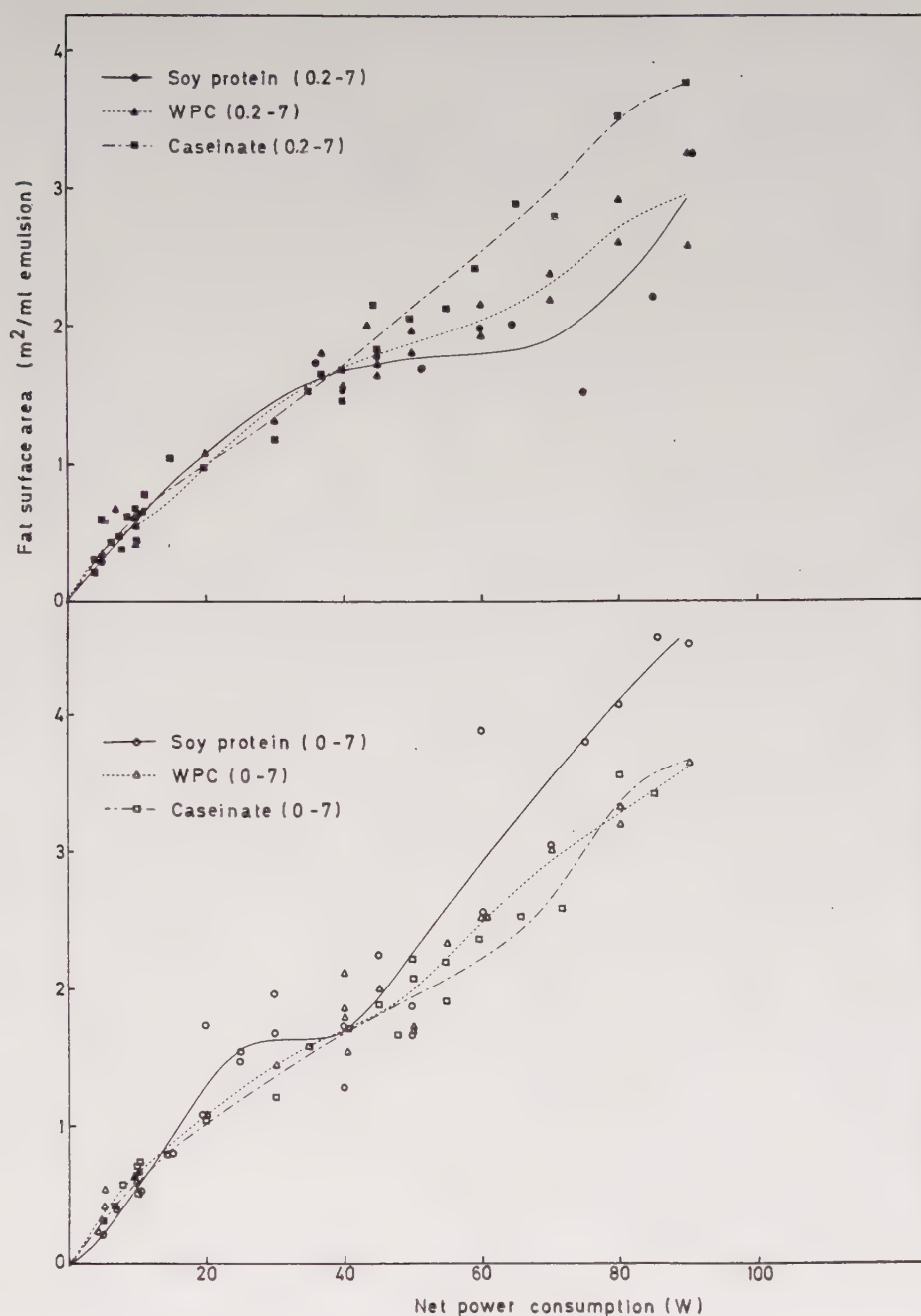


Figure 1 Fat surface area of protein stabilized emulsions as a function of net power consumption after emulsification with a valve homogenizer using 10 passes through the recirculating system.

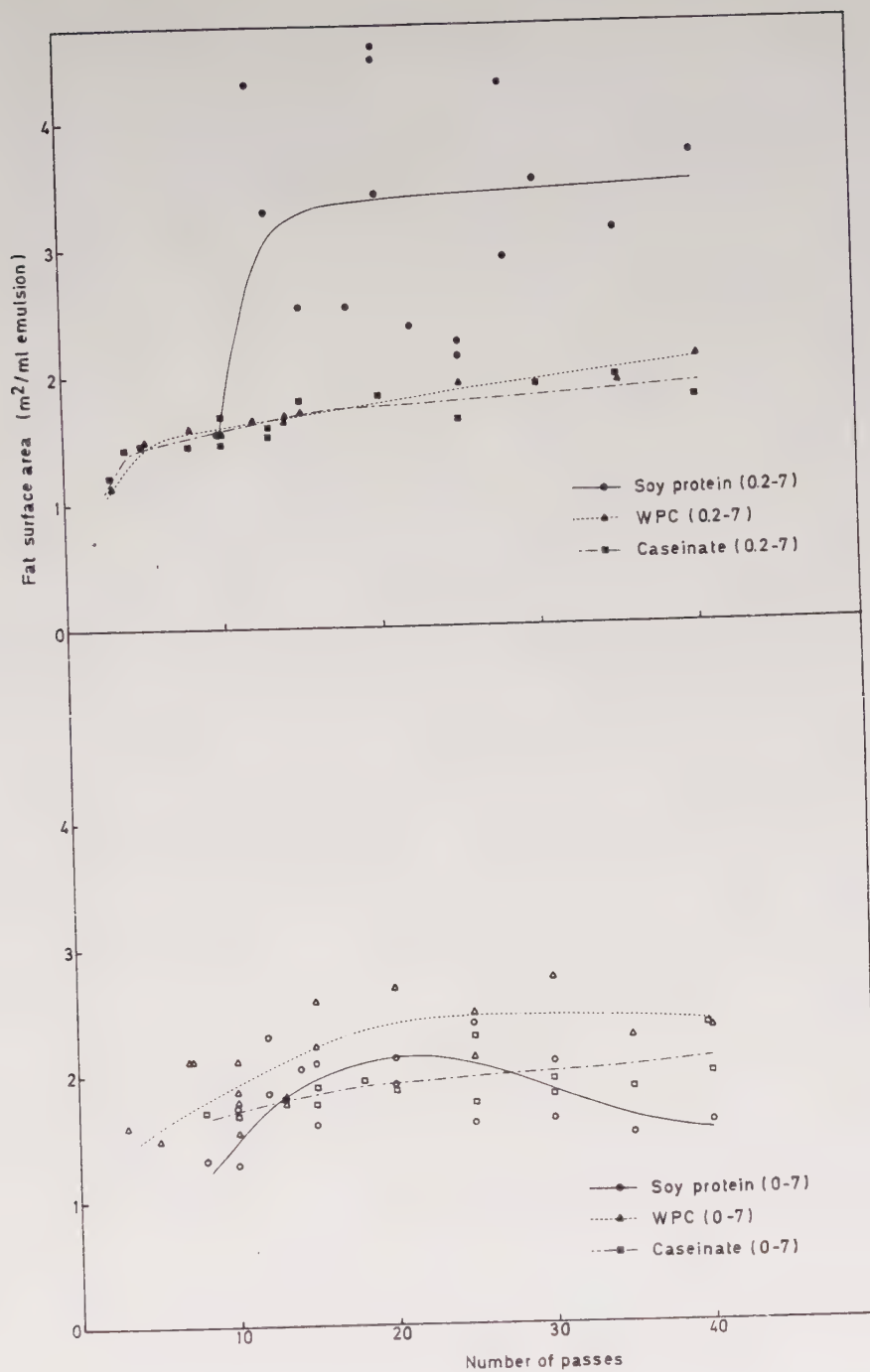


Figure 2 Fat surface area of protein stabilized emulsions as a function of number of passes after emulsification with a valve homogenizer at a constant power supply of 40 W.

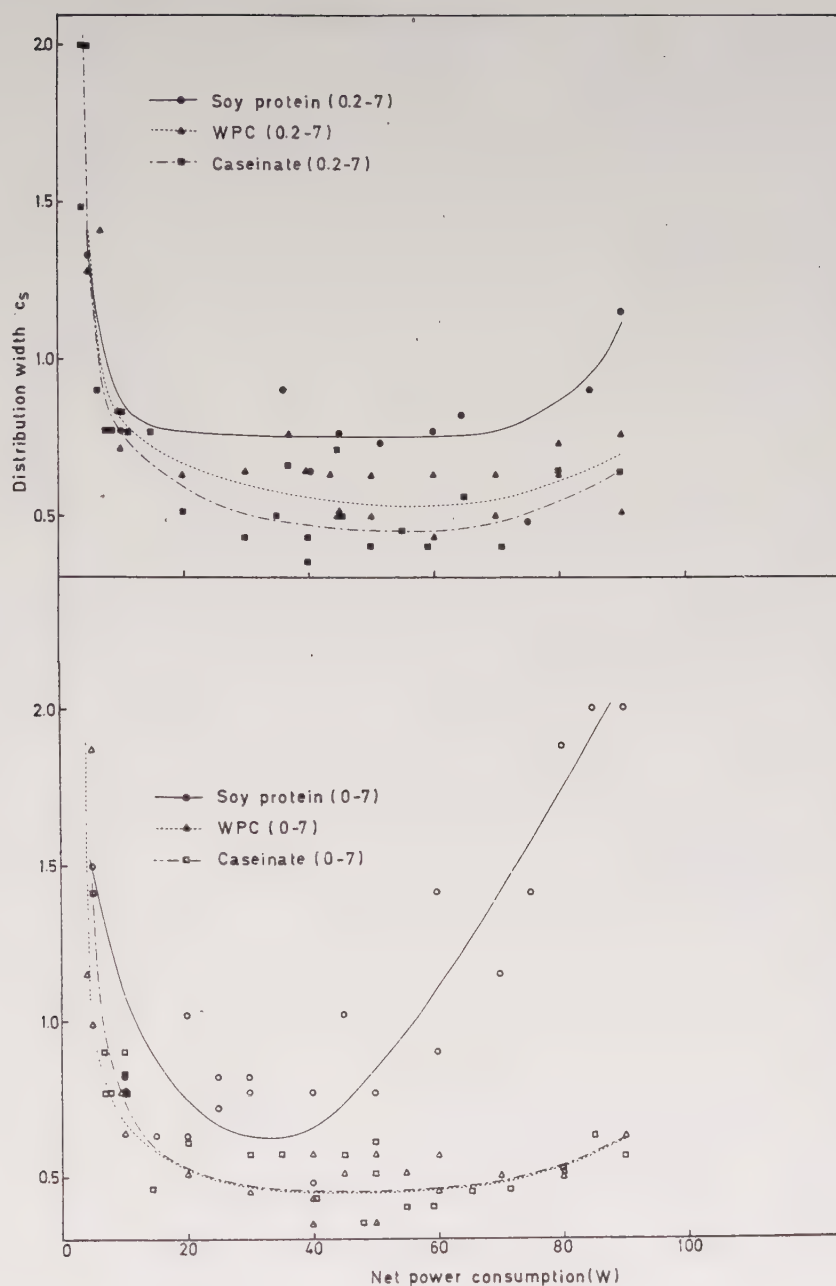


Figure 3 Distribution width, c_s , of the protein stabilized emulsions as a function of net power consumption, on emulsification with a valve homogenizer using 10 passes through the recirculating system.

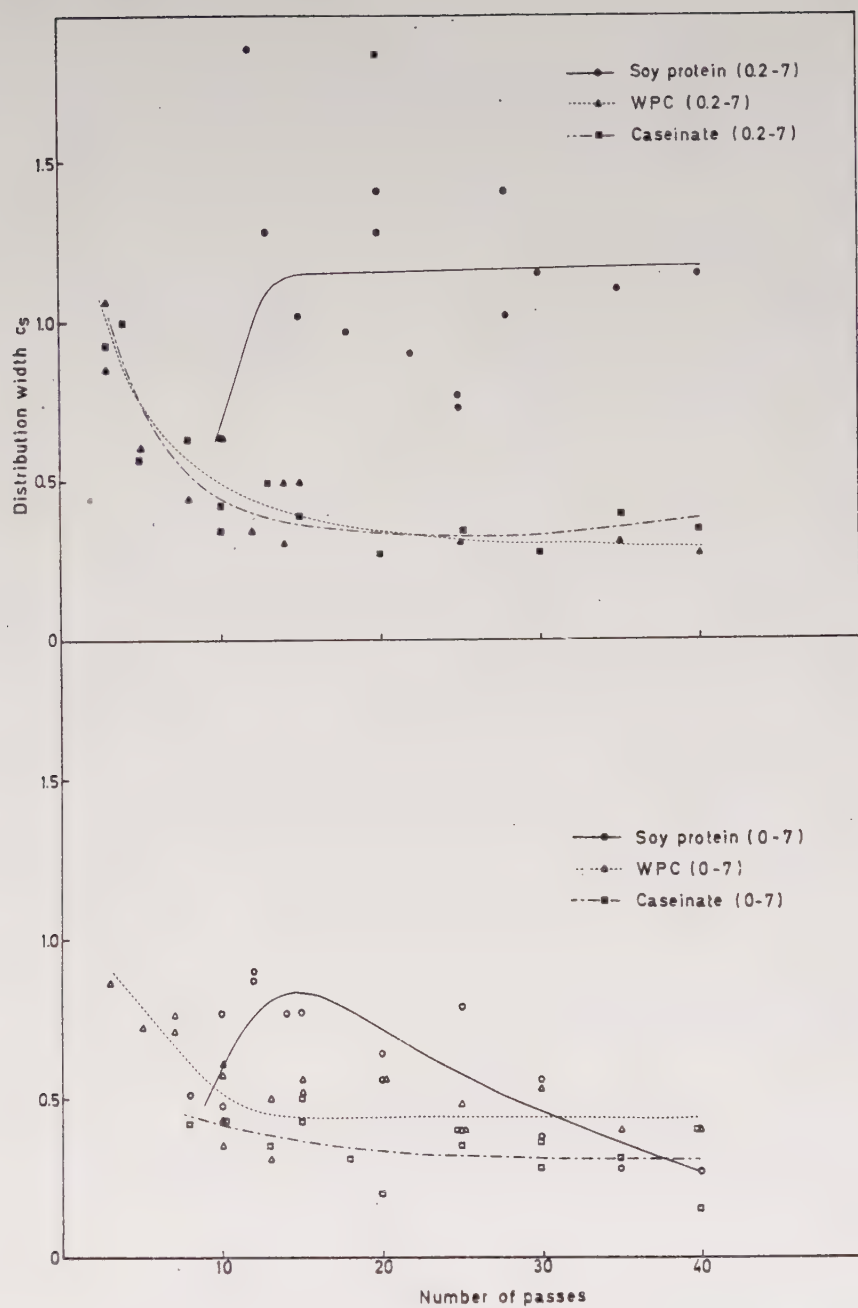


Figure 4 Distribution width, c_s , of the protein stabilized emulsions as a function of number of passes, on emulsification with a valve homogenizer at a constant power supply of 40 W.

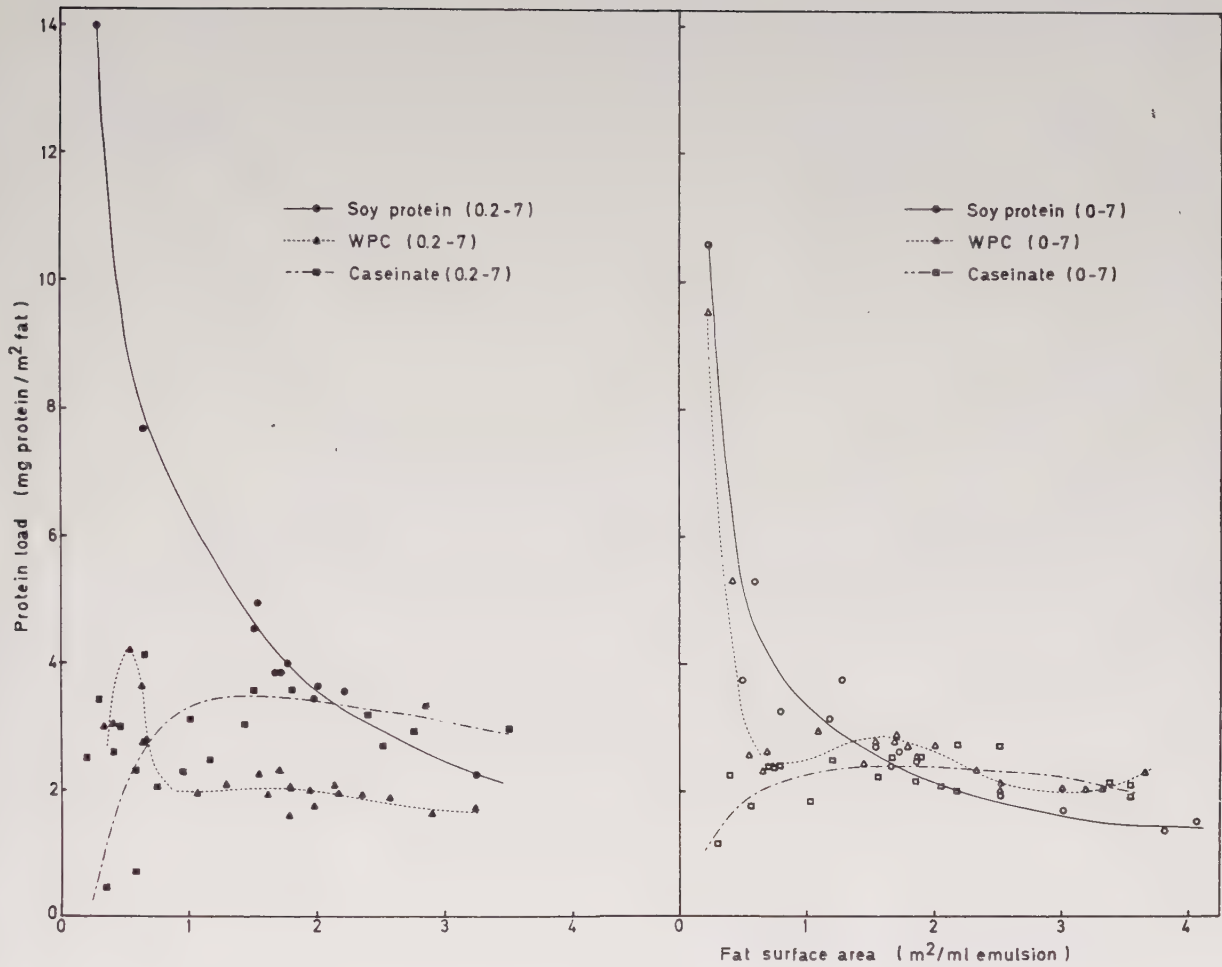


Figure 5 Protein load of the protein stabilized emulsions as a function of the fat surface area created during variation of power supply at a constant number of passes (10).

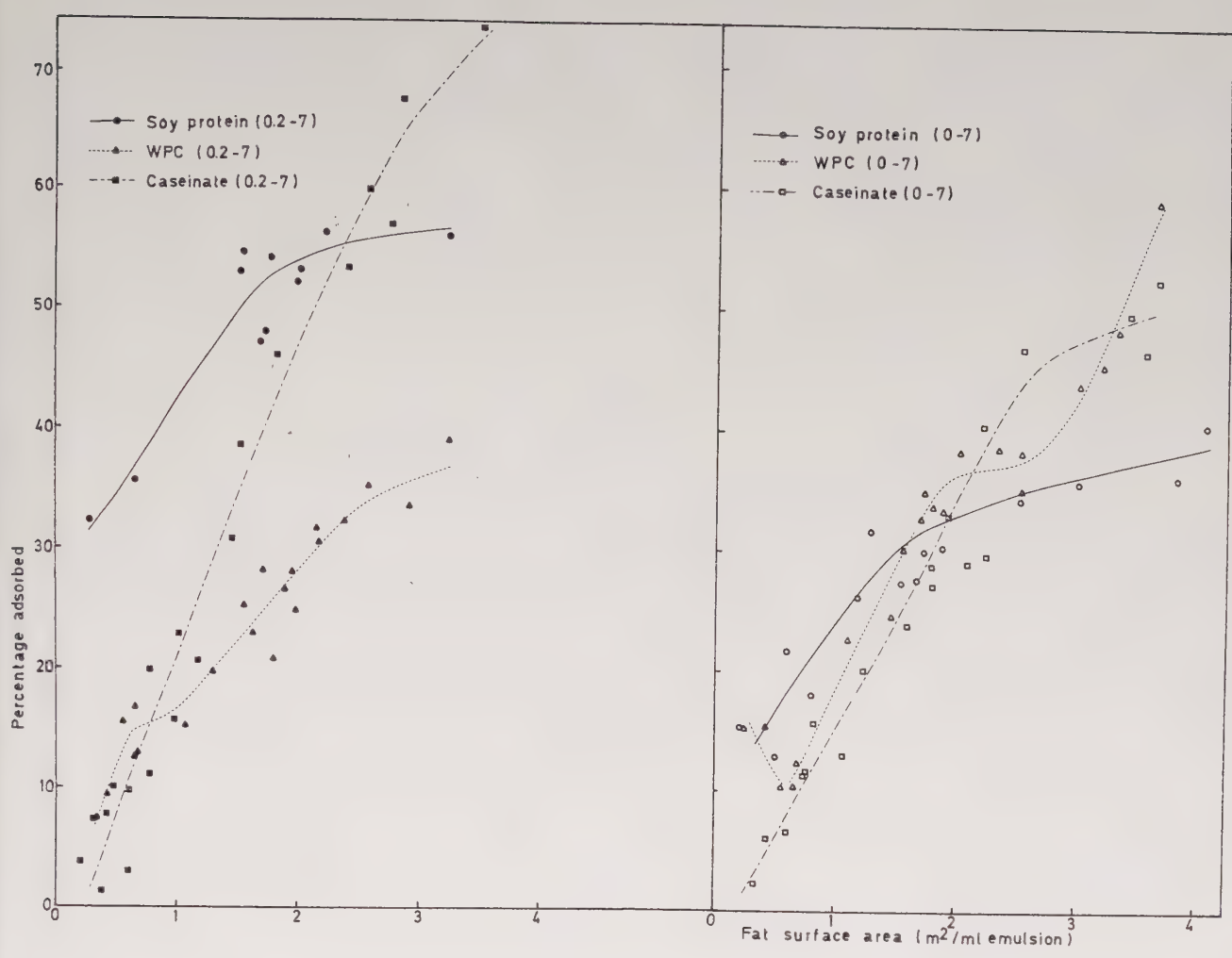


Figure 6 Percentage adsorbed protein from the bulk to the interface of the protein stabilized emulsions as a function of the fat surface area created during variation of power supply using a constant number of passes (10).

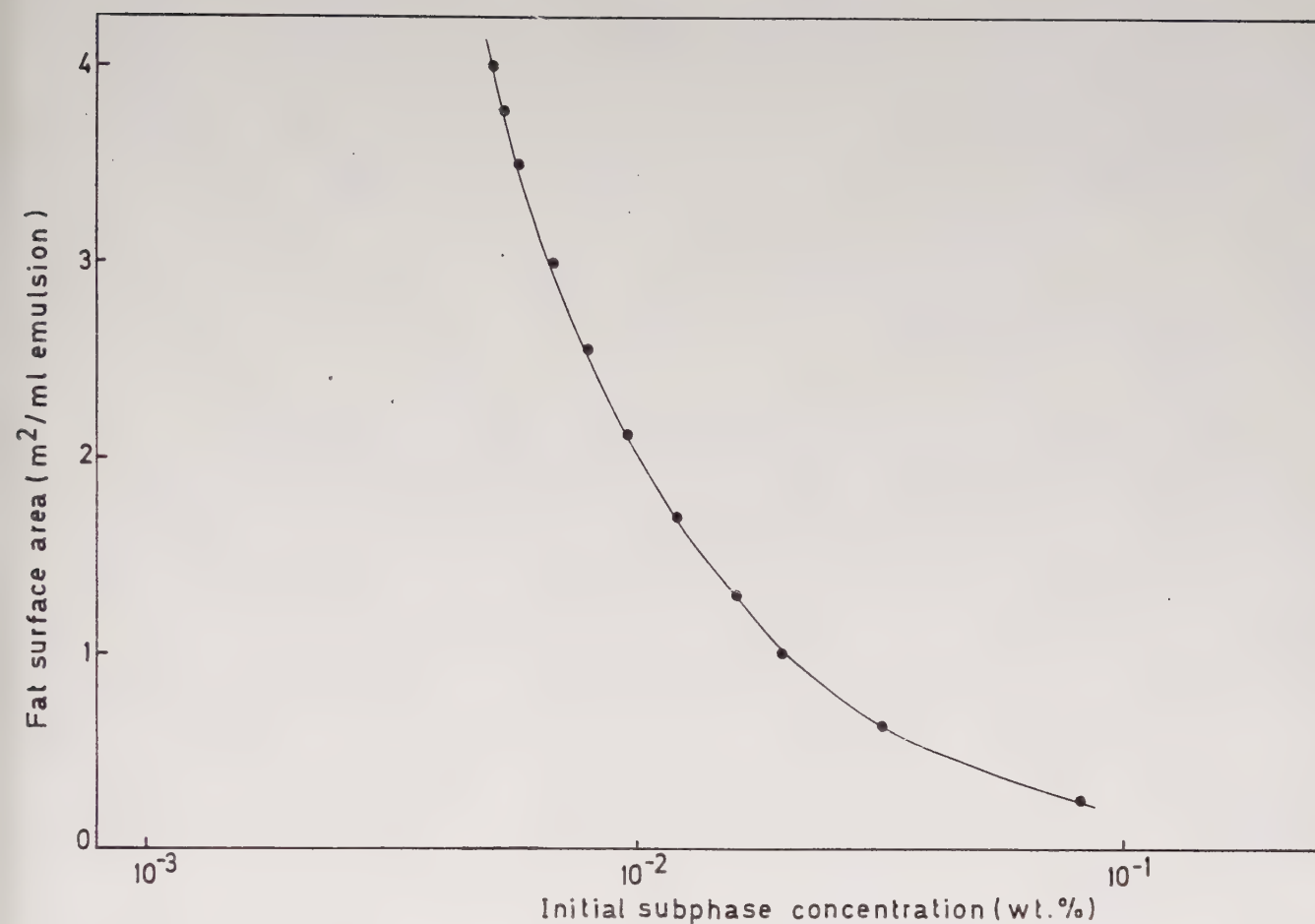


Figure 7 The correspondence between the interfacial area of an emulsion made with an initial protein content of 2.5% (w/w) in the continuous phase and the initial subphase concentration during the interfacial tension measurements according to ref.²⁰, if the amount of protein available in the bulk before adsorption in relation to the interface is set equal.

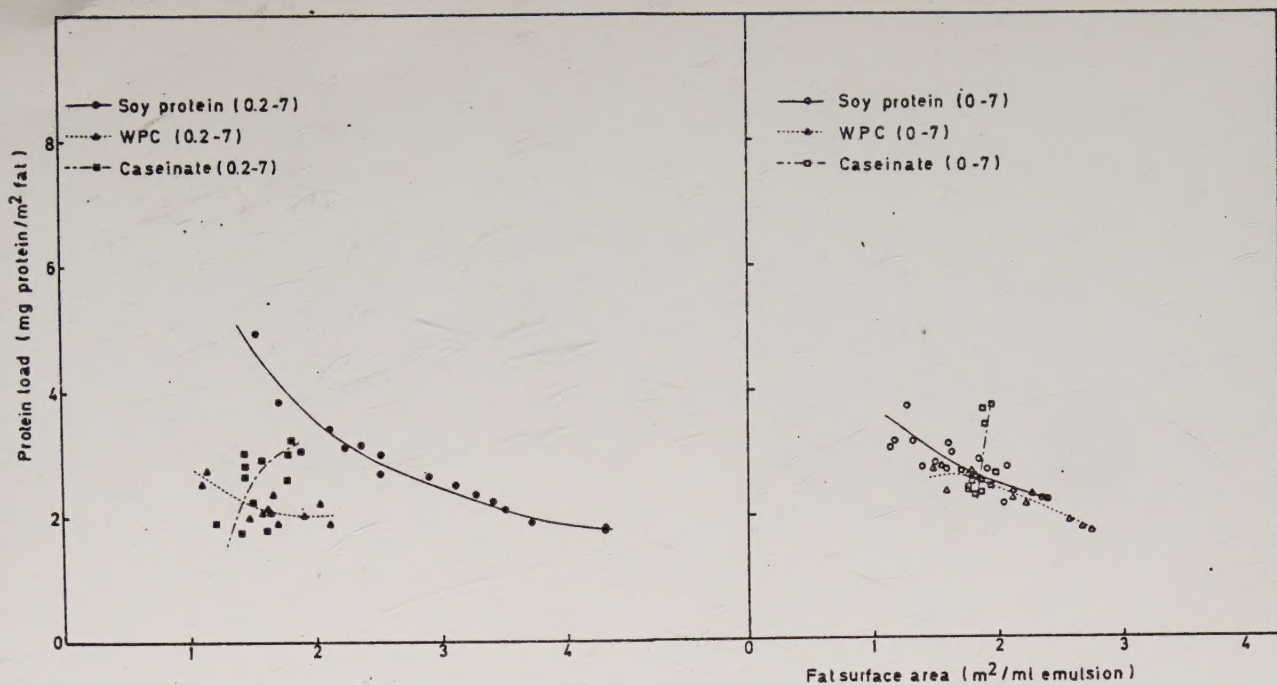


Figure 8 Protein load of the protein stabilized emulsions as a function of the fat surface area created during variation of number of passes at a constant power supply of 40 W.

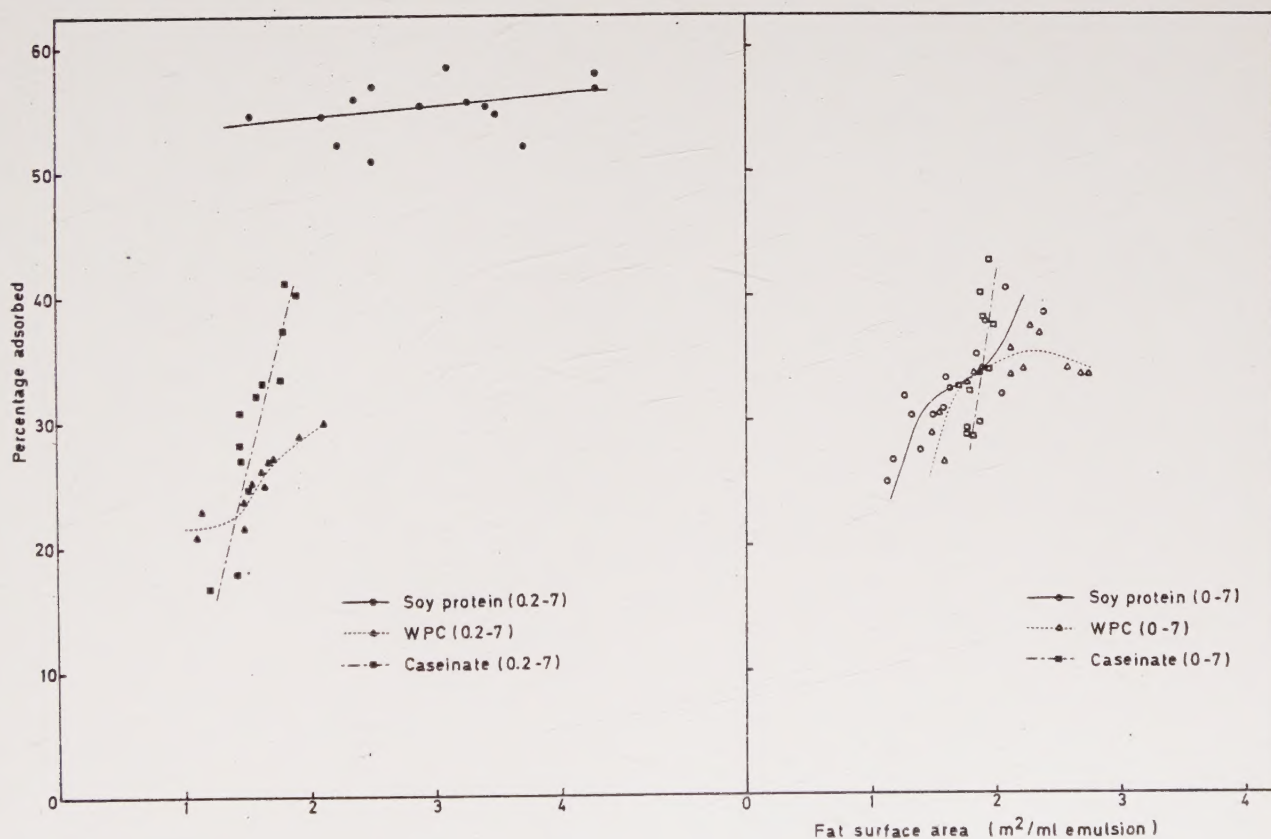


Figure 9 Percentage adsorbed protein from the bulk to the interface of the protein stabilized emulsions as a function of the fat surface area created during variation of number of passes at a constant power supply of 40 W.

